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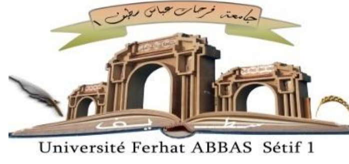

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AMARI Salima

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JURY:

Chair:	**ARRAR Lekhmici**	**Pr. UFA Setif 1**
Supervisor:	**CHAREF Noureddine**	**Pr. UFA Setif 1**
Examiners:	**GHERRAF Noureddine**	**Pr. UAB Oum El Bouaghi**
	ZELLAGUI Amar	**Pr. UAB Oum El Bouaghi**
	AKKAL Salah	**Pr. UFM Constantine1**
Invited:	**KARBAB Ahlem**	**MCB. UFA Setif 1**

Laboratory of Applied Biochemistry

"بسم الله الرحمن الرحيم"

DEDICATION AND ACKNOWLEDGEMENTS

Dedication

Praise be to **Allah** alone, my Lord, thank you until you are satisfied, and thank you if you are satisfied.

To my dear three persons: Said, Yamina and Laid;

My dear husband **Said**, for his endless love, support, help and encouragement;

The most precious person in existence and the most beautiful and sweetest name that my mouth says, my dear mother **Yamina;** may Allah protect you and reward you and prolong your life;

The one who was the cause of my existence and the one who has favor on me that I cannot return as long as I live, my dear father **Laid**;may Allah prolong your life and best reward you;

My family and my husband's family, without exception;

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To all of you, I dedicate this humble work, hoping that it will contribute to enlightening the knowledge arena with useful information in our field of research.

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AMARI S, KARBAB A, ARRAR L and CHAREF N. Phytochemical study and biological properties of *Erica arborea*. L leaves and *Zygophyllum album*. L aerial parts extracts. 1st International Conference of Biodiversity in the Service of Biotechnologies, Mila, Algeria. **2020**.

AMARI S, KARBAB A, ARRAR L and CHAREF N. Evaluation of phenolic compounds content and biological activities of *Artimisia herba alba* and *Rosmarinus officinalis* ethanolic extracts. 1st National Conference of Technological Innovations in Sustainable Agriculture Service, Mila, Algeria, **2021**.

AMARI S, KARBAB A and CHAREF N. Fractionation of phenolic extracts of plant origin: research of natural biopesticides of *pectobacterium*. 1st International Conference of Natural Substances, Biotechnologies and Innovations, Mila, Algeria. **2021**.

ABSTRACTS

ملخص

الخلنج هي شجيرة صغيرة لها استعمالات تقليدية متعددة في منطقة بجاية بالجزائر حيث أنها معروفة بكونها مدرة للبول، تعالج الإمساك والتهابات المسالك البولية وحصوات الكلى والتهابات متعددة. إن هذه الدراسة تهدف إلى التحقق من فعالية الاستخدامات العلاجية للأجزاء المختلفة من النبتة بالإضافة إلى تقييم مختلف الأنشطة البيولوجية كالنشاطات المضادة للأكسدة، مضادات الجراثيم، مضادات الالتهاب (في الكائنات الحية)، التأثيرات المسكنة ومضادات حصوات المسالك البولية. أظهرت البيانات المأخوذة من الدراسة أن 28 ٪ من السكان المحليين الذين استخدموا هذه النبتة في الطب الشعبي. يحتوي الخلنج على مركبات كيميائية تم الكشف عنها في المستخلصات المختلفة للأوراق والزهور والتي تتمثل في البوليفينول والفلافونويد والتانينات القابلة للتحلل والكينون الحر والأنتراكينون والصابونين والتربينويدات. وبينت جميع مستخلصات الأوراق (LE)، ومستخلصات الأزهار (FE) وجود كميات معتبرة من البوليفينول والفلافونويد والعفص المكثف. كانت القيمة الإجمالية من مادة البوليفينول والفلافونويد في مستخلص أسيتات الإيثيل (EaEs) للأوراق والأزهار، والتي تراوحت من 649.38 إلى 944.55 ميكروغرام مكافئ حمض الغاليك / ملغ من المستخلص الجاف ومن 65.31 إلى 67.15 ميكروغرام من مكافئ كيرسيتين / ملغ من المادة الجافة، على الترتيب. بينما وجدت أعلى قيمة من العفص المكثف في المستخلص الخام للأوراق (CrE) والمستخلص المائي (AqE) للأزهار. كما أظهرت نتائج تحليل HPLC-MS أيضًا وجود epicatechin وpalmitic acid وkaempferol-3-*O*-glucoside في مختلف المستخلصات الميثانولية المائية المختبرة. و بين مستخلص الأسيتات ايثيل EaE للأزهار و المستخلص الخام CrE للأوراق نشاط ضد الأكسدة معتبر من خلال اختبار إزاحة جذر DPPH (IC_{50} = 17.72 ميكروغرام / مل) واختبار القدرة الارجاعية (IC_{50} 0.291 ميكروغرام / مل)، على التوالي. أظهرت المستخلصات المختبرة فاعلية بدرجات مختلفة مضادة للجراثيم. وبالفعل مستخلص الأزهار أظهر نشاطًا جيدا مضادًا للجراثيم، خاصةً ضد *P. aeruginosa* و *M. luteus*. التقييم السمي بين أن (CrEs) التي تم تناولها عن طريق الفم لم تسبب الموت أو تغير في سلوك الفئران المعالجة. بالإضافة إلى ذلك، CrE و DecE بينت تأثير مضاد للتورم ضد وذمة الأذن الناتجة عن كزيلان أو زيت كروتون في الفئران. علاوة على ذلك تساهم CrEs في تقليل التقلصات البطنية بشكل كبير بجرعة 500 مغ / كغ في نموذج التلوي الناجم عن حمض الخل وأظهر أيضًا نشاطًا كبيرًا مضادًا لحصوات مجرى البول في فحوصات التنوي والتجميع. علاوة على ذلك، تؤكد نتائجنا الاستخدام الطبي لهذه النبتة في الطب التقليدي وأن مستخلصات أوراق وزهور نبتة الخلنج يمكن استخدامها كموارد طبيعية مهمة كمضادات حصوات المسالك البولية، مضادات الالتهابات، مضادات الجراثيم، ومضادات الأكسدة ومسكنات.

الكلمات المفتاحية: الخلنج، دراسة عرقية، سمية، نشاطات بيولوجية، بوليفينول.

ABSTRACT

Erica arborea L. is a small shrub widely known in Algeria's region of Bejaia by its uses for diuretic purposes as well as to treat constipation, urinary tract infections, kidney stones and inflammation. This study aimed to investigate medicinal uses of the different plant parts of *E. arborea* and to examine their different biological activities such as antioxidant, antibacterial, anti-inflammatory (*in vivo*), analgesic and anti-urolithiatic. The data of an ethnopharmacological survey showed that 28 % of local habitants used this plant in folk medicine. *E. arborea* contains various compounds such as polyphenols, flavonoids, hydrolyzable tannins, quinones, anthraquinones, saponins and terpenoids in both leaves and flowers extracts. All the tested extracts (LE) and (FE) showed an appreciable total content of phenolic compounds, flavonoids and condensed tannins. The highest total phenolic and flavonoids contents was present in the ethyl acetate extracts (EaEs) of leaves and flowers ranging from 649.38 to 944.55 µg gallic acid equivalent/mg dry extract and 65.31 to 67.15 (µg quercetin equivalent/mg dry extract), respectively. While, the highest condensed tannins content was present in the crude extract (CrE) of leaves and aqueous extract (AqE) of flowers. Results of HPLC-MS analysis also revealed the presence of epicatechin, palmitic acid, and kaempferol-3-*O*-glucoside in extracts. The EaE of flowers and CrE of leaves exhibited the better antioxidant activity by 2, 2-diphenyl-l-picrylhydrazyl (DPPH) scavenging test (IC_{50} =17.72 µg/mL) and reducing power test (IC_{50} = .029 µg/mL), respectively. Furthermore, results showed that the extracts exhibited an antibacterial power at variable degrees against all the pathogens. Indeed, the *E. arborea* flower extract showed maximum antibacterial activity especially against *P. aeruginosa* and *M. luteus*. The results of the toxicity assay showed that CrEs are safe, where no deaths or changes were noted in the behavior of treated mice. Moreover, CrE and decoction extract (DecE) caused a considerable anti-edematogenic effect in the xylene and croton oil-induced ear edema in mice dose-dependent in two experimental models. However, CrEs significantly reduced abdominal contractions at a dose mg/kg500 in the acetic acid-induced writhing model and exhibited also significant anti-urolithiatic activity in nucleation and aggregation assays. In addition, the results support the use of *E. arborea* in folk medicine and the leaves and flower extracts of *E. arborea* could be used as important resources of natural anti-urolithiatic, anti-inflammatory, antibacterial, and antioxidant and analgesic agents.

Keywords: *Erica arborea* L, ethnopharmacological survey, toxicity, biological activities, phenolic compounds.

Résumé

Erica arborea L. est un petit arbuste largement utilisé dans la région de Béjaia en Algérie à des fins diurétiques ainsi pour traiter la constipation, les infections urinaires, les calculs rénaux et les inflammations. Cette étude visait à étudier l'utilisation médicinale de différentes parties d'*E. arborea* et d'évaluer leurs différentes activités biologiques telles que antioxydantes, antibactériennes, anti-inflammatoires (*in vivo*), analgésiques et anti-urolithiatiques. Les données d'une enquête ethnopharmacologique ont montré que 28 % des habitants locaux utilisaient cette plante en médecine populaire. *E. arborea* contient des composés phytochimiques dans différents extraits testés de feuilles (LE) et fleurs (FE) tels que les polyphénols, les flavonoïdes, les tanins hydrolysables, les quinones, les anthraquinones, les saponines et les terpénoïdes. Tous les extraits testés de (LE) et (FE) ont montré des teneurs appréciables en polyphénols, flavonoïdes et tanins condensés. Les teneurs totaux en polyphenols et flavonoïdes les plus élevées ont été trouvées dans les extraits d'acétate d'éthyle (EaEs) de feuilles et fleurs, allant de 649.38 à 944.55 µg d'équivalent d'acide gallique/mg d'extrait sec et de 65.31 à 67.15 µg d'équivalent de quercétine/mg d'extrait sec, respectivement. Alors que la teneur en tanins condensés la plus élevée a été trouvé dans l'extrait brut de feuilles et l'extrait aqueux de fleurs. Les résultats de l'analyse HPLC-MS ont également révélé la présence d'épicatéchine, d'acide palmitique et de kaempférol-3-*O*-glucoside dans les extraits méthanoliques testés. L'extrait d'acétate d'éthyle (EaE) de fleurs et l'extrait brut de feuilles (CrE) ont montré le meilleure activité antioxydante par le test de piégeage du radical 2,2-diphényl-l-picrylhydrazyle (DPPH) (IC_{50} = 17.72 µg/mL) et le test de pouvoir réducteur (IC_{50} = 0.29 µg/mL), respectivement. Les extraits testés ont exhibé un pouvoir antibactérien variable vis-à-vis les agents pathogènes. En effet, l'extrait de fleur d'*E. arborea* a montré une meilleure activité antibactérienne, particulièrement vis-à-vis de *P. aeruginosa* et *M. luteus*. L'évaluation de la toxicité a montré que les extraits bruts (CrEs) administrés par la voie orale n'ont pas induit ni la mort ni le changement dans le comportement des souris traités. De plus, CrE et l'extrait de decoction (DecE) ont provoqué un effet anti-œdématogène considérable vis-à-vis de l'œdème de l'oreille induit par le xylène ou l'huile de croton chez les souris ; cet effet était dose-dépendant dans les deux modèles expérimentaux. De plus, les CrEs ont provoqué la réduction de manière significative les contractions abdominales à une dose mg/kg500 dans le modèle de contorsion induit par l'acide acétique et ont montré également une activité anti-urolithiatique significative dans les tests de nucléation et d'agrégation. De plus, nos résultats soutiennent l'utilisation d'*E. arborea* en médecine traditionnelle et les extraits de feuilles et fleurs d'*E. arborea* pourraient être utilisés comme des resources importantes des agents naturels anti-urolithiatiques, anti-inflammatoires, antibactériens, antioxydants et analgésiques.

Mots clés *: Erica arborea* L, étude ethnopharmacologique, toxicité, activités biologiques, composés phénoliques.

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ABBREVIATIONS

ABBREVIATIONS

5-LOX	5-lipoxygenase
AP	Aerial part
AqE	Aqueous extract
ATCC	American type culture collection
BHT	Butylated hydroxytoluene
BW	Body weight
CE	Catechin equivalent
ChE	Chloroform extract
COX-2	Cyclooxygenase-2
CrE	Crude extract
DecE	Decoction extract
DMSO	Dimethylsulphoxide
DPPH	1, 1-diphenyl-2-pycrylhydrazyl
DW	Dry weight
EaE	Ethyl acetate extract
Ext	External
FE	Flowers extract
FG	Functional group
GAE	Gallic acid equivalents
HPLC-DAD-MS	High-performance liquid chromatography-diode array detector-mass
HPLC-MS	High-performance liquid chromatography-mass spectrometry
IC_{50}	50% inhibitory concentration
IL-1	Interleukin-1
IL-10	Interleukin-10
IL-6	Interleukin-6
iNOS	Inducible nitric oxide synthase
Int	Internal
LE	Leaves extract
MAPK	Mitogen-activated protein kinase
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
NF-κB	Nuclear transcription factor Kappa-B
NSAID	Non-steroidal anti-inflammatory drugs
PGE_2	Prostaglandins E_2
PKC	Protein kinase C
PLA_2	Phospholipase A_2
QE	Quercetin equivalents
ROS	Reactive oxygen species
SD	Standard deviation
SEM	Standard error of mean
TC	Condensed tannins
TNF-α	Necrosis factor-alpha
TPA	12-O-tetradecanoylphorbol-13-acetate.
TPC	Total phenolic contents

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INTRODUCTION

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Our health depends on well functioning metabolic processes, hemoestasis and functioning repair mechanisms in our body. Free oxygen radicals play a major role in the proper functioning of our body, which in some cases can lead to oxidative stress, which is the cause of a number of dysfunctions in the human body (Much *et al.*, 2021). It has extensively been demonstrated that strong and complex interconnections occur between oxidative stress, inflammatory responces and other pathologies (Allegra, 2019; Ly *et al.*, 2021). Antibiotics and chemical synthetic drugs cause various disadvantages and side effects. However resistance of bacteria to the available antibiotic is growing rapidly and the available antibacterial agent also cause diverse adverse reactions such as hypersensitivity and immunosuppression (Stanković *et al.*, 2016). The synthetic antioxidants can present unwanted side effect and some have been suspected of being responsible for liver damage and carcinogenesis. Also, the prolonged utilization of non-steroidal anti-inflammatory drugs (NSAIDs) can produce deterious effects on the gastrointestinal tract (Karbab *et al.*, 2021).

Many countries encouraging the screening programs of herbs used in traditional remedies in order to authenticate their pharmacological properties. Plants with medicinal properties play an increasingly important role in the primary health care for their functions on diseases prevention and treatment. Indeed, these plants long used as safe, effective and sustainable resources of natural products (Tajner-Czopek *et al.*, 2020). Natural products long used in the development of natural-derived agents to treat numerous chronic disorders linked with antioxidant potentials (Chaves *et al.*, 2020), anti-urolithiatic effects (Ly *et al.*, 2021), inflammatory responses and pathogenic bacterial infections (Alinjhad *et al.*, 2016). In this respect, medicinal and aromatic plants that exhibit antioxidant, anti-inflammatory, antiurolithiatic and antibacterial effects may have a wide range of medicinal applications (Mueller *et al.*, 2015; Ly *et al.*, 2020). The biological activities of medicinal plant extracts, obtained from stems, leaves, flowers, or aerial parts has been extensively examined by different research groups (Yaici *et al.*, 2019; Pham *et al.*, 2020).

Erica arborea L is a typical shrub belonging to the Ericaceae family. This plant grows in the Mediterranean Basin, East Africa and is widespread in North Africa. *Erica arborea.* L. is commonly called white heather (Amezouar *et al.*, 2013) and locally as Khlenj (Yaici *et al.*, 2019). In folk medicine, Pavlovic *et al.* (2014) mentioned the Ericaceae species to treat numerous pathologies of urinary infections and wounds.

Within this context, *E. arborea* has been traditionally used by local healers in several countries, such as Algeria (Guendouze-Boucheffa *et al*., 2015; Yaici *et al*., 2019). The leaves, flowers, and flowering branches have shown numerous activities such as diuretic, antiseptic, and laxative (Ay *et al*., 2007). Furthermore, the leaves and stems of the plant were externally mashed to treat insect bites, and the flowers were prepared by decoction for treating urinary tract infections, inflammation, and hypotension problems (Darias *et al*., 2001). In North Algeria, the flowers of *E. arborea* L. have been widely recommended for treating bedwetting and kidney stones in both infusion and decoction forms (Eddaikra et al., 2019).

Previous studies indicated that extracts from different parts of *E. arborea* exhibit bio activities such as antioxidant (Amezouar *et al*., 2013; Kivçak *et al*., 2013), antimicrobial (Guendouze-Bouchefa *et al*., 2015; Yaici *et al.*, 2019), analgesic (Akkol *et al*., 2007), and anti-inflammatory effects (Akkol *et al*., 2007; Amezouar *et al*., 2013, Amroun *et al*., 2021). In this regard, Amroun *et al*. (2021) demonstrated that the *E. arborea* L. decoction extracts contain significant amounts of phenolic compounds, which may be responsible for the use of this plant in folk medicine in the treatment of inflammatory diseases.

In light of the previous data, the present study aimed to evaluate the ethnopharmacology study, phytochemicals constituents, toxicity study, and pharmacological activities of *Erica arborea* L. (leaves and flowers). The current study was based on the following parts:

- ✓ Evaluate the ethnopharmacological survey about *Erica arborea* L. from region of Bejaia, especially Djebel Tadergount.
- ✓ Extraction procedures, qualitative and quantitative assessment of phytochemical components in different extracts.
- ✓ HPLC-MS phytochemical profiling, toxicity study and examination of the antioxidant, antibacterial, anti-inflammatory, analgesic and anti-urolithiatic potentials of *Erica arborea* L. extracts to provide scientific bases for its use in traditional medicine.

LITTERATURE REVIEW

1. Natural products

Natural substances formed by plants have provided a continuous source of medications (Santos *et al*., 2012). Several studies have identified herbs as sources of development for the majority of currently used pharmacological drugs and potential for the future discovery of new agents against a range of disorders with a range of biochemical mechanisms of action (Selamoglu, 2018). Also, natural products from these plants are easily available, efficient, safe, inexpensive and rarely accompanied by side effects (Razmavar *et al*., 2014). These natural products produced by plants and microorganisms in response to external stimuli including nutritional changes (Bereksi *et al*., 2018) and may be divided into two broad categories, primary and secondary metabolites. Secondary metabolites originates from primary metabolites include the carbohydrates, amino acids, and lipids (Kumar and Goel, 2019).

1.1. Phenolic compounds and classification

Phenolic compounds are the most parts of secondary metabolites presents in natural sources with enormous structural diversities and significant importance (Alara *et al*., 2021). They can be described as compounds that contain a phenol moiety. Also, the phenol itself is a benzene ring that is substituted with a hydroxyl group and it systematic name is hydroxybenzene (Al Mamari, 2021). Thus, phenolic compounds can be divided into several groups (Figure 1) (Othman *et al*., 2019).

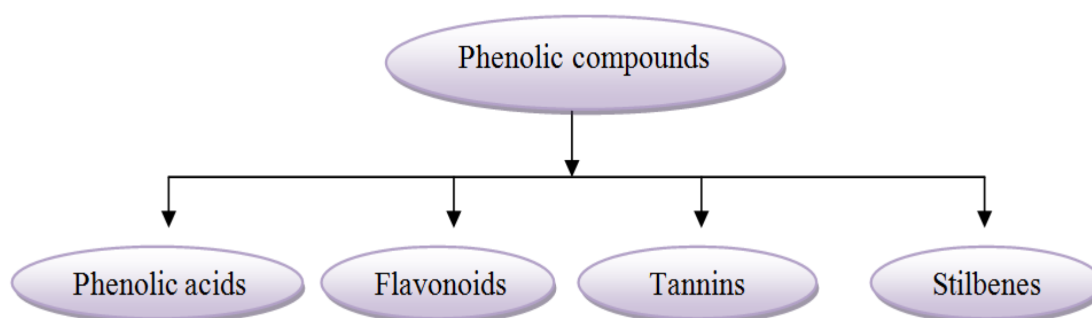

Figure 1: Main classes of phenolic compounds (Alara *et al*., 2021).

1.2.1. Phenolic acids

Phenolic acids are present in free and bound forms (Zhang *et al*., 2022). Phenolic acids can be subdivided into two types (Figure 2), hydroxybenzoic acid, with C_6–C_1 structure, such as gallic and

vanillic acid, and hydroxycinnamic acids, with a 3-carbon side chain (C_6–C_3), that consist essentially of coumaric, caffeic, and ferulic acid (Miklasinska-Majdanik *et al.*, 2018).

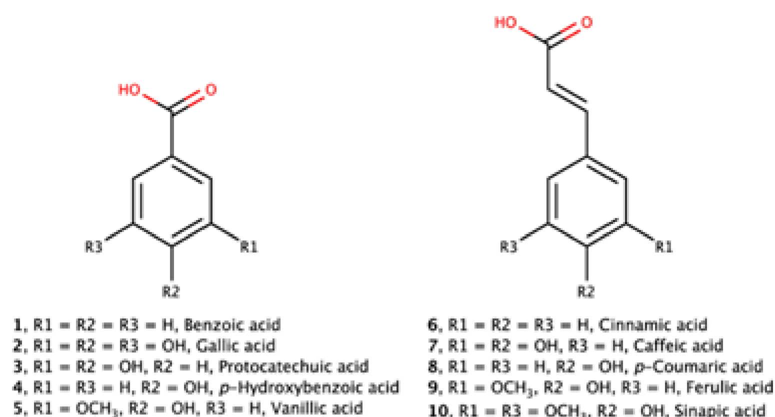

Figure 2: Representative examples of phenolic acids (Zhang *et al.*, 2022).

1.2.2. Flavonoids

All flavonoids display a standard structure of two phenyl rings (A and B) connected to a heterocyclic ring C, which containing the embedded oxygen. This carbon structure can be abbreviated as C6-C3-C6. These compounds frequently display hydroxylation at the 5, 7 positions at A ring and oxidation at positions 3′, 4′ or 3′,4′,5′ at B ring due to their biosynthesis routes (Shamsudin *et al.*, 2022). The family members of flavonoids are commonly found in nature and have a significant chemical diversity mainly because of the changes in B- and C-ring locations, degree of hydroxylation, oxidation, and saturation of ring C (Al Mamari, 2021). Also, flavonoids are classified according to their chemical structures into flavan-3-ol, flavanones, flavones, flavonols, isoflavones and anthocyanidins (Shamsudin *et al.*, 2022), as shown in Figure 3. The numbering system of a basic flavonoid structure is also illustrated in Figure 3.

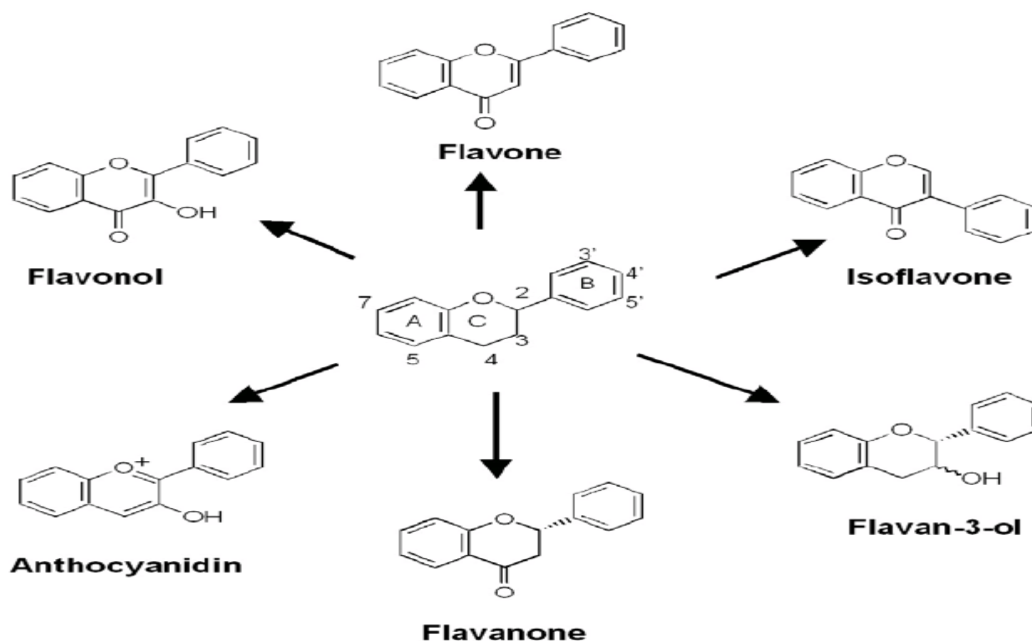

Figure 3: General structure and various classes of flavonoids (Nishiumi *et al*., 2011).

1.2.3. Other polyphenols

In addition to this diversity, polyphenols are present in plant tissues mainly as glycosides, associated with various organic acids or as complex polymerized molecules with high molecular weights, such as tannins (Daglia, 2012).Tannins are commonly subdivided into 2 groups: hydrolysable and condensed tannins (Figure 4). The hydrolysable tannins contain a central glucose core in an esterified form with gallic acid. The condensed tannins or proanthocyanidins they are either oligomers or polymers of flavan-3-ol bonded through the interflavan carbon bond (Naumann *et al*., 2017).

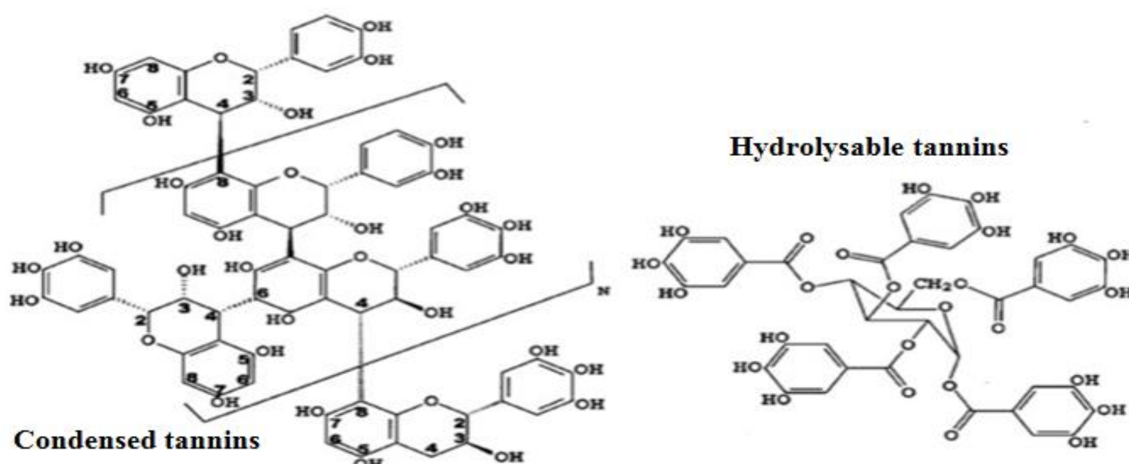

Figure 4: Representatives structures of condensed and hydrolysable tannins (Zak *et al*., 2019).

2. Phenolic compounds and biological activities

2.1. Antioxidant activity

2.1.1. Free radicals and oxidative stress

The oxidation is essential chemical reaction to many organisms for the production of energy for biological processes and can produce reactive oxygen species (ROS) (Selamoglu, 2018). However, the ROS are often over-produced under some diseases or stress conditions, resulting in oxidative stress (Luis *et al*., 2021; Siddeeg *et al*., 2021). Oxidative stress is defined as an increase in cellular free radicals generation and decrease in antioxidant levels (Pehlivan *et al*., 2021) which create an imbalance between cellular defense and pro-oxidant (Bedolla *et al*., 2013).

The free radicals in the forms of ROS are continuously generated in the human cellular systems (Figure 5). However, these cells are also well equipped with an efficient endogenous antioxidant system that is composed of both enzymes and other non-enzymatic molecules (Pharm *et al*., 2020; Siddeeg *et al*., 2021). Enzymatic antioxidants will be breaking down and be removing free radicals by converting dangerous oxidative products to hydrogen peroxide (H_2O_2) and then to water, the process had multistep and had the presence of cofactors such as copper, zinc, manganese, and iron. On the other hand, non-enzymatic antioxidants will interrupt free radical chain reactions (Pharm *et al*., 2020).

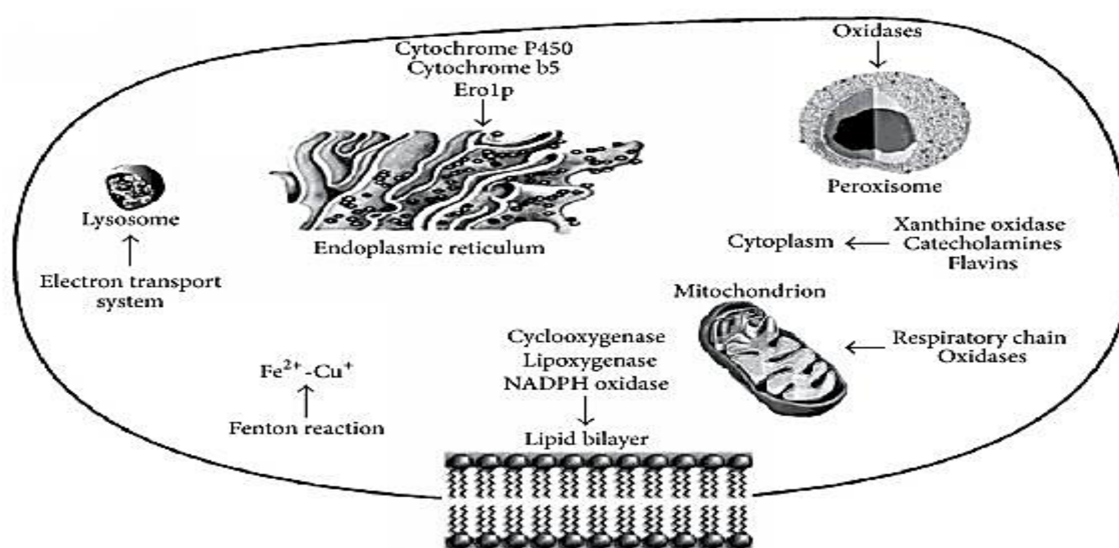

Figure 5: Cellular sources of ROS production (Venditti et al., 2015).

Other exogenous factors such as smoking, ionizing radiation, pollution, organic solvents, and pesticides are able to attack nucleic acids, proteins, enzymes, and other small molecules causing loss of structure and function (Siddeeg *et al.*, 2021) and leading to the development of several chronic and sub-chronic disorders including neurodegenerative diseases (Parkinson, Alzheimer), cardiovascular diseases, liver disorders, myocardial infarction, viral infections, inflammation, cancer, diabetes, autoimmune pathologies, and digestive system disorders (Almada-Taylor *et al.*, 2018; Selamoglu *et al.*, 2020). However, most diseases induced by oxidative stress appear with age because the aging decreases antioxidant defenses and increases mitochondrial production of free radicals (Wu *et al.*, 2021).

2.1.2. Effect of phenolic compounds on oxidative stress

Natural antioxidants present in plants are tremendously important substances that possess the ability for inhibiting or preventing the harmful consequences caused by free radical induced oxidative stress (Almada-Taylor *et al.*, 2018; Pharm *et al.*, 2020; Phuyal *et al.*, 2020). Among these molecules, phenolic compounds forms the most important group of biologically active molecules in plants, used therapeutically as antioxidants. The potent antioxidant activity of phenolics compounds and flavonoids compounds are associated with a lower incidence of cardiovascular diseases, cancer, diabetes and neurodegenerative diseases (Aryal *et al.*, 2019). Their effectiveness in the inhibition of oxidative processes in foods is related to two main approaches including their reducing and non-reducing effects (Bujor *et al.*, 2016).

Plant phenolic compounds act as reducing agents by the electron or hydrogen-donating property (Mahdi-Pour *et al.*, 2012; Aryal *et al.*, 2019; Phuyal *et al.*, 2020). In biological systems, the ions can be involved in cyclic redox reactions, generating ROS (Platzer *et al.*, 2021). Thus, phenolic compounds are known to act as antioxidants by inhibiting the prooxidative action of metal ions by a chelation action. Phenolic compounds are also implied in the reduction of ROS through the inhibition of prooxidative enzymes such as xanthine oxidase, lipooxygenase, NADPH oxidase and myeloperoxidase (Bujor *et al.*, 2016). Furthermore, some phenolics such as quinones act as a source of stable free radicals and bind irreversibly with proteins leading to its loss of function (Othman *et al.*, 2019).

The common responsible factor for antioxidant activity for extracts of plants is the presence of phenolic compounds which were correlated to their chemical structures (Table 1) (Chen *et al.*,

2002; Žuvela *et al.*, 2019; Sakurai *et al.*, 2021). Thus, they can facilitate the donation of hydrogen atoms and electrons from their hydroxyl groups and their aromatic ring (Koldas *et al.*, 2015; Chaves *et al.*, 2020). Considering the influence of the number of hydroxyl groups on the molecule, the mean value increased with increasing number of hydroxyl group (Platzer *et al.*, 2022). Additionally, the OH groups are located at different positions on phenolic molecule is also responsible for the observed large variations in the trends of anti-oxidative properties (Nam *et al.*, 2017).

Table 1: Structure – antioxidant activity relationship of flavonoids (Platzer *et al.*, 2022).

Antioxidant agent	**Related activity**	**Structure-antioxidant activity relationship**
Reactive species scavenging		
Baicalin, quercetin, heliosin, and hyperoside,	$OH^{\cdot}$ scavenging activity	Importance of positions of hydroxyl groups Importance of otho-dihydroxy group on the A-ring (on 5,6 positions) Importance of meta-dihydroxy on the A-ring and ortho-OH in the B-ring (on 3 , 4 positions)
Morin, taxifolin, kaempferol, and galangin	DPPH· scavenging activity	Importance of ortho-dihydroxy on the B-ring (on 3 ,4 position) and/or with a OH on the C-ring at the position 3
Quercetin and rutin	Lipid peroxidation activity	Presence of OH group additional in the benzene ring
Chelators of transition metal ions		
Myricetin and quercetin, catechin	Chelating activity of Cu^{2+}	5-OH-4-carbonyl site.8 3 -OH-4'-OH (orthocatechol)

2.2. Antibacterial activity

2.2.1. Antibiotic drugs and mechanism of bacterial resistance

Antibiotics drugs are produced by micro-organisms and derivatives of semi-synthetic or entirely synthetic products, capable of selectively inhibiting of certain metabolic pathways of bacteria, without exerting toxic effects on organisms superiors (Sanchez, 2015). Third-generation beta-lactam antibiotics are commonly used in urinary tract infections by bacteria and indiscriminate antibiotic use resulted in the development of resistance to one or multiple antibiotics that give a severe challenge upon disease treatment or even treatment failure beside other adverse effects on the liver and bone marrow (Allami *et al*., 2020).

Mechanisms of antibiotic resistance in bacteria include the following Figure 6: Efflux of the antibiotics from the bacterial cell through efflux pumps (Gorniak *et al*., 2019); prevention of interaction of the drug with the target by changing membrane potential (Singh et al., 2016); alteration of the antibiotic target site through genetic mutations and overproduction of them by gene amplification (Hughes and Andersson, 2017); presence of enzymes produced by bacteria that inactivate the antibiotics through their hydrolysis and their chemical modification by phosphorylation (Munita and Arias, 2016).

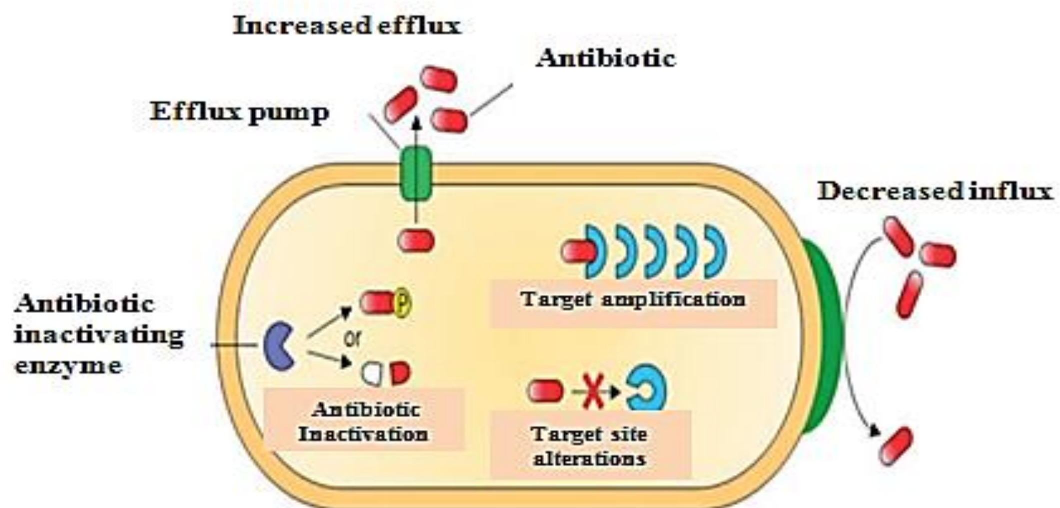

Figure 6: Diagrammatic representation of antibiotic resistance mechanisms utilized by bacteria (Alav *et al*., 2018).

2.2.2. Effect of phenolic compounds on bacteria

Phenolic compound known by altering microbial cell permeability, leading to its damage (Razmavar *et al.*, 2014; Osonga *et al.,* 2019; Yuan *et al.*, 2021). The bacterial membrane damage by catechins and other flavonoids is caused by binding to the lipidbilayer and it resulted the inability of the bacteria to secrete toxins (Lee *et al.*, 2011; Górniak *et al.*, 2019; Motallebi *et al.*, 2020). Thus, hydrophilic flavonoids can interact at the membrane surface and provide protective actions against efflux pump and biofilm formation of deleterious agents (Awolola *et al.*, 2014; Xie *et al.*, 2015). Phenolic acid such as *p*-coumaric, caffeic, and ferulic acid induced higher ion leakage and a significant influx of protons into the bacterial cells (Campos *et al.*, 2009; Panda and Duarte-Sierra, 2022). The Disruption of membrane integrity can directly or indirectly cause metabolic dysfunction and finally lead to bacterial death (Hartmann et al., 2010).

Many enzymes responsible for either the bacterial growth or virulence factor might lose their activity in response to phenolic compounds (Razmavar *et al.*, 2014. Furthermore, the presence of tannins and flavonoids caused a toxic effect and an inhibition of different types of enzymes and transporter proteins found in the cell membrane and inside cell (Razmavar *et al.*, 2014). They have also shown inhibitory activity against different kinds of lactamases produced by bacteria, which are the key enzymes that disable the common antibiotics (Xie *et al.*, 2015). Other targets of phenolic compounds are binding to cell wall proteins, complexing with metal ions and others (Li *et al.*, 2015; Samsonowicz *et al.*, 2017; Paz *et al.*, 2018; Othman *et al.*, 2019).

The structural diversity of polyphenols is immense and their antimicrobial potency produced against microorganisms is attributed to its structural characteristics (Bitchagno *et al.*, 2015 ; Adamczak *et al.*, 2019; Farahadi *et al.*, 2019; Osonga *et al.*, 2019; Osorio *et al.*, 2021; Yuan *et al.*, 2021). The relationship of the antimicrobial activity of plant polyphenols is classified into four types: (1) number of functional groups (FG), (2) position of FG, (3) type of FG, and (4) presence of C2=C3 double bond ((Panda and Duarte-Sierra, 2022). Furthermore, the structure- antibacterial activity relationship of some polyphenols against bacteria is illustrated in the following Table 2.

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Table 2: Summary of structure–antibacterial activity relationship of some polyphenols against bacteria.

Antibacterial agent	Structure –activity relationship	Reference
Nombre of functional groups		
Naringenin	Three additional OH groups.	(Echeverría *et al.*, 2017)
Caffeic acid	Additional –OH group on the phenolic ring	(Kepa *et al.*, 2018)
5-hydroxyflavanones 5-hydroxyisoflavanones	Three additional OH groups at the 7, 2′ and 4′ positions	(Stapleton *et al.*, 2004)
Position of functional groups		
Quercetin	OH group at position 3 in the C ring	(Wu *et al.*, 2013)
Baicalein	OH groups at the positions 5, 6,7 in the A ring	(Yadav *et al.*, 2013)
luteolin, quercetin, and myricetin	OH groups at the positions 3’,4’ positions in the B ring	
Galangin	Two OH groups located on ring A and the absence of polar groups on ring B	(Echeverría *et al.*, 2017).
Chalcones	Isoprenoid or methoxy groups at positions 3’ , 5’ , and 2’ of ring A	(Omosa *et al.*, 2016).
Sophoraflavanone	Isogeranyl at C-8 and OH at 3, 2’ and 4’ at A and B rings	(Oh *et al.*, 2011).
Quercetin3-O-[α-L-Rhamnopyranosyl-(1 → 6)-β-D glucopyranoside]	Disaccharide group at the groups instead of the OH group at position 3	(Tebou *et al.*, 2017)
Type of functional groups		
Balcalein , myricetin	Pyrogallol structure and three hydroxyl groups	(Xie *et al.*, 2017)
Tangeritin, nobeltin	Methoxyl group at C8 in the A ring	(Liu *et al.*, 2012)
Naphthoquinone	Without chlorine	(Bouarab-Chibane *et al.*, 2019)
Epigallocatechingallate	Gallic or galloyl moieties	(Kuete *et al.*, 2011)
Caffeic acid	Longer alkyl esters side chain	(Andrade *et al.*, 2015)
C2=C3 double bond		
Naringenin	Saturation of C2=C3 double bond	(Xie *et al.*, 2017)

2.3. Anti-inflammatory activity

2.3.1. Inflammatory mechanism

The simplest form of acute inflammatory response are triggered to prevent the body against external aggressions (skin burns, tissue injury) and harmful microbial, viral or fungal attack (Villegas-Aguila *et al*., 2020), thus, this response can be divided in to two general steps: initiation and resolution (Okoli *et al*., 2007). Tissue swelling and pain, resulting by sequential release of various several mediators including serotonin and histamine from activated mast cells (Ouédraogo *et al*., 2012; Buckley *et al*., 2014), manifest initiation phase. The origin of this event is a cascade of release of chemotactic factors such as chemokines which helping to leukocytes migration (Botting and Botting, 2000). Thus, these events are followed by increase in blood vessel permeability and blood viscosity (Buckley *et al*., 2014). The main events in resolution are cessation of polynuclear leukocytes cells (PNN) and macrophages clearance of debris.

The acute inflammation becomes harmful noxious when prolonged over times, resulting in chronic age-related diseases, including arthritis, rheumatoid, cancer, diabetes, atherosclerosis, alzheimer's diseases (Rea *et al*., 2018; Villegas-aguilar *et al*., 2020). The inflammatory response is a land where lipid mediators such as prostaglandins and leukotrienes and other inflammatory mediators uncovered (Serhan *et al*., 2015). Products and enzymes of arachidonic acid metabolism involved in the inflammatory process are shown in Figure 7.

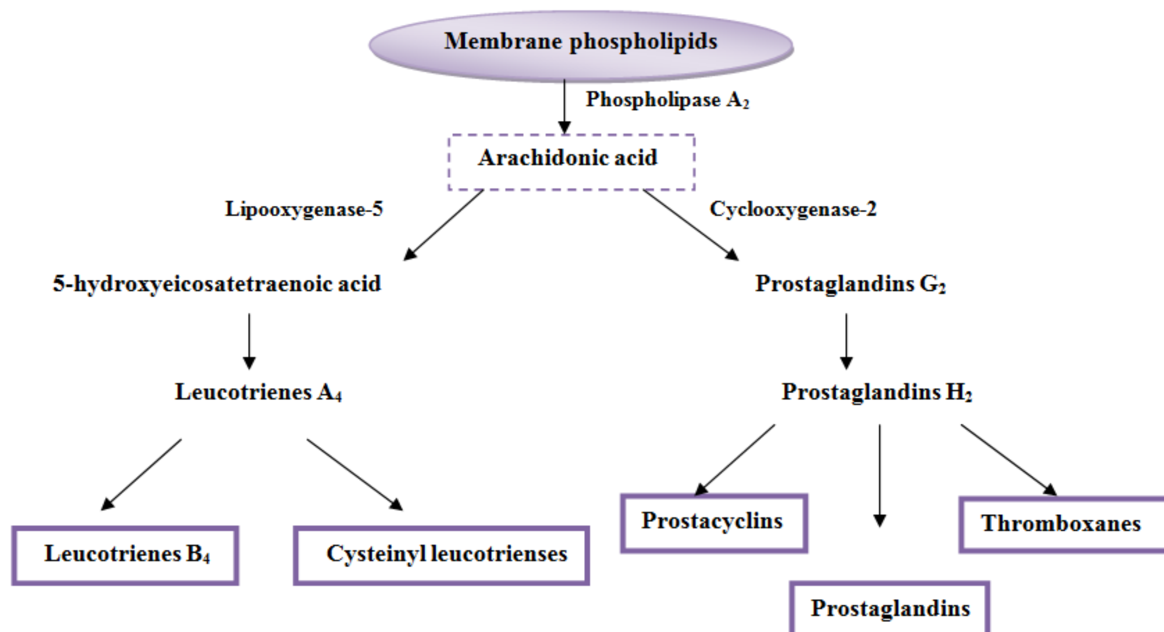

Figure 7: Products and enzymes of arachidonic acid metabolism involved in the inflammatory process (Bewaji *et al*., 2013).

2.3.2. Effect of phenolic compounds on inflammation

Numerous studies established the effect exerted by the phenolic compounds isolated from plants and vegetal oil on the main metabolic pathways involved in inflammatory process in vivo an in vitro assay (Càrdeno *et al.*, 2014; Sogo *et al.*, 2015; Lee *et al.*, 2006). Moreover, phenolic compound of *Theobroma cocoa* has shown in mouse with 12-O-tetracanoylphorbol-13-acetate (TPA) induced mouse skin (Lee *et al.*, 2006; Villegas-aguilar *et al.*, 2020).

A number of mechanisms were also related to these phenolic compound, which are confirmed by previous discusses such as inhibition of increase vascular permeability, inhibition of neutrophil migration into inflamed tissues, stimulation of lymphocytes accumulation, suppressing the pro-inflammatory cytokines release (Okoli and Akah, 2004; Okoli *et al.*, 2007; Amro *et al.*, 2013). The flavanone called pinocembrin (5,7-dihydroxyflavanone) from *pinus caribaea* was identified as anti-inflammatory agent, which contains a 2-phenyl-benzopyran-4-one skeleton (Sinyeue *et al.*, 2021). Summarized in Table 3 are some selected reviews and reports related to the effects of polyphenols on inflammation pathways.

Table 3: Effects of some natural products on inflammation pathways.

Natural products	Medicinal plants	Inflammation pathways	References
Maslinic acid 4,5-dicaffeoylquinic Porphyran	*Olea europea* *Ainsliaea fragrans* *Porphyra yezoensis*	Inhibiting nuclear factor-kappa B (NF-κB) activated pathway	(Huang *et al.*, 2011; Chen *et al.*, 2015 ; Isaka *et al.*, 2015)
Delphinidin 3-sambubioside,	*Hibiscus sabdariffa*	Down regulate the NF-κB pathway and extracellular signal-regulated kinase (ERK) signaling through suppression of numerous inflammatory mediators	(Sogo *et al.*, 2015)
Hydroxytyrosol	*Olea europea L*	Reduces the inflammatory mediators including iNOS, nitric oxide (NO), interleukin -1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α)	Yonezawa *et al.*, 2018)
Tannins	*Citrus microcarpa*	Preventing leukotrienes B4 and prostaglandins (PGE-2) biosynthesis by phospholipase A2 blocking	(Alinejhad *et al.*, 2016).
Dihydromyricetin	*Ampelopsis grossedentata*	Inhibits the proinflamatory cytokines and increases the production of anti-inflammatory cytokine interleukin-10 (IL-10)	(Hou *et al.*, 2015)

2.4. Urolithiatic activity

2.4.1. Stone formation mechanism

Nephrolithiasis is known a significant pathology within the world population, with grave medical consequences, during a patient's life (Chen *et al.*, 2018; Ammor *et al.*, 2020). It is caused by a wide variety of causes metabolic, nutritional, microbial infectious and drug-related (Ammor *et al.*, 2020). Thus, uric acid excretion rate, urine pH, hypercalciuria and hyperphosphaturia are also considered as risk factors of calcium oxalate kidney stones (Ahmed *et al.*, 2018).

Calcium oxalate kidney stones is characterized by mineral deposition in urinary system and the calcium oxalate monohydrate are most preponderant stones deposited (González et al., 2020), which formed generally by binding free oxalate to calcium (Ahmed *et al.*, 2018; Ammor *et al.*, 2020). Calcium oxalate crystals has been shown to have affinity to cells membranes of renal epithelial and therefore leads to the disorder of the normal activities of these cells, caused changes in gene expression, impairment of mitochondrial function, forming ROS and thus, decreased cell viability. Figure 8 illustrates the mechanism of stone formation (Aggarwal *et al.*, 2010).

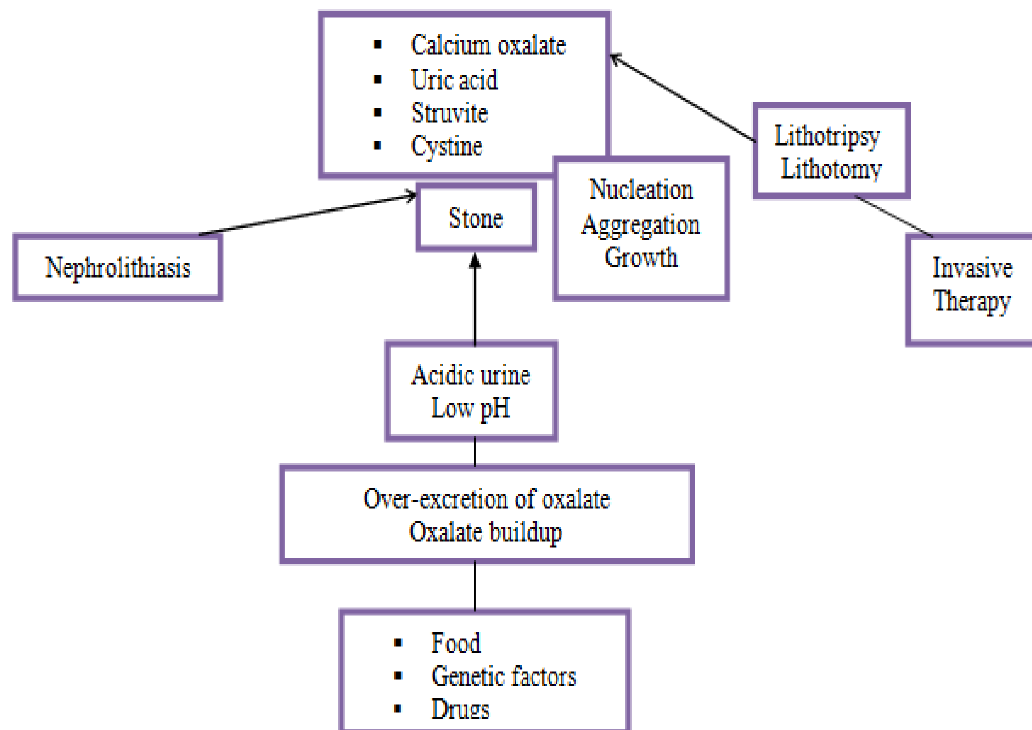

Figure 8: Mechanism of kidney stone formation (Ahmed *et al.*, 2018).

2.4.2. Effect of phenolic compounds on urolithiasis

In addition, Standard chemicals drugs such as allopurinol, citrate, cystone and thiazide diuretics are used to prevent and treat urolithiasis (Ahmed *et al.*, 2018). The present-day, due to the harmful effects of these medications and lithotripsy within the body, includes acute kidney damage, decreased renal function and hypertension (Ammor *et al.*, 2020), the use of many plants in the treatment of kidney stones has been estimated by researchers. Thus, in traditional medicine, antiurolithiatic plants have been prescribed to eliminate kidney stones, dissolve kidney stones or prevent stone formation (Mordi *et al.*, 2020).

These polyphenols blocked the formation of calcium oxalate particles and prevented the calcium oxalate crystal aggregation and their growth as well as their deposits in renal cells (Gupta and Kanwar, 2018; Zeng *et al.*, 2018; Ly *et al.*, 2021). The antioxidant, anti-inflammatory and diuretic activities of polyphenols are commonly related to the calcium oxalate calculi prevention (Ahmed *et al.*, 2016; Moreno *et al.*, 2021). The diuretic action increases the quantity of fluid going pass through the kidneys as a consequence flush out the deposits (Shukla *et al.*, 2017) and the antioxidant action play an important role in the avoidance of calculi formation (Ahmed *et al.*, 1018).

3. Toxicity

In traditional medicines, the users used various medicinal plants and needed testing it to valid their safety or possible potential toxicity (Subramanian *et al.*, 2018). The potential toxicity of medicinal plants may be related to over doses or chemical composition of the plant in question and their safety is based on their long usage in the treatment of diseases and also their natural origin. Indeed, the combination of substances of several plants medicinal in traditional preparations makes it difficult to predict the toxic effect of mixture (Subramanian *et al.*, 2018). Their toxic effects are evaluated by the dose administered, weight changes, modification of biochemical parameters of the blood, histology of certain organs and the observed mortality rate (Karbab, 2020).

4. *Erica arborea* L

The Ericaceae are a large cosmopolitan family (Amezouar *et al.*, 2013), represented by 4250 species grouped into 124 genera includes Erica (Christenhusz and Byng, 2016). The genus of Erica includes more than 700 species (Akkol *et al.*, 2008). This genus is represented in the Algerian flora by five taxa where *E. arborea.* L specie is distinguished (Yaici *et al.*, 2019).

4.1. Taxonomy

According to Cronquist (1988), *Erica arborea* L. is classified as follows:

- **Kingdom** : plantae
- **Division** : Spermaphytes
- **Sous-division** : Angiosperm
- **Class** : Eudicots
- **Order** : Ericales
- **Family** : Ericaceae
- **Genus** : Erica
- **Specie** : *Erica arborea.* L

4.2. Botany and ecology

Erica arborea L. is one of the dominant heathers in African Mediterranean region and can reach generally 40 to 60 cm in wide and 1 to 4 m in height, with erect and tight stems. The youngest stems are whitish and the thin leaves are linear, resembling needles (Bessah *et al.*, 2014; Guendouze *et al.*, 2015). It is characterized by deep root system, with a large diameter (Aubert, 1978) and the fruit in the form of capsule, which are surrounded by a persistent corolla (Gizaw et al., 2013). *Erica arborea* is white heather because of the color of its bell-shaped flowers, which formed during spring from March to April (Figure 9). The plant of *Erica arborea* is widely presents in Australia, South Africa, Portugal and Canary Islands (Amezouar *et al.*, 2013). In Africa, it is widespread in Morocco, Tunisia and Algeria in high altitude of the Aurès and the Ksour mountains as well as the Djurdjura massif (Ait Youssef, 2006, Guendouze *et al.*, 2015). *Erica arborea* colonizing, especially the semi-arid regions and growing on siliceous soils (Amezouar *et al.*, 2013).

Figure 9: Leaves and flowers of *Erica arborea* L. (Amroun, 2021).

4.3. Chemical composition

The specie of *E. arborea* contain several phytochemicals especially phenolic compounds, flavonoids, condensed tannins, coumarins essential oils and terpenoids (Ait Youcef, 2006; Bessah *et al*., 2014; Suna *et al*., 2018). In addition, the methanolic extract of *Erica arborea*. L lead the isolation of newly constituents such as and quercitrin and (-)-epicatechin (Ay *et al*., 2007). *E. arborea* L plant is tested also by Zengin *et al*., (2019) using LC- Mass Spectrometry analysis, providing 72 phenolic compounds, 21 and 42 of which have been identified as phenolic acid and flavonoids (Zengin et al., 2019). In earlier study of Amroun *et al*. (2021) using high-performance liquid chromatography- diode array detector-mass spectrometry (HPLC-DAD-MS), the Algerian samples of the same specie revealed that they contain several phenolics including myricetin pentoside, quercetin-3-O-glucoside, myricetin-3-O-rhamnoside, quercetin-3-O-pentoside, quercetin-3-O-rhamnoside, chlorogenic acid, p-coumaric acid and 5-O-p-coumaroylquinic acid (Amroun *et al*., 2021).

According to the studies of Bessah *et al*. (2014), the composition of the essential oil of Algerian *E. arborea* was determined by gas chromatography coupled with mass spectrometry. It is characterized by a high content of palmitic acid and 9, 12, 15-octadecatrice-1-ol. Thus, the other components are present in small proportions such as β-fenchyl alcohol, β-caryophyllen, β-bourbonen, eugenol, ionol and geranylaceton. In this species, the presence arbutoside, tannins has also been reported (Ay *et al*., 2007).

4.4. Traditional use and biological activities

E. arborea is classified as plant used traditionally to manage many diseases because of their health benefits and pharmacological activities (Zengin *et al*., 2019). Previous studies shown potent biological activities such as antimicrobial (Guendouze-Boucheffa *et al*., 2015; Yaici *et al*., 2019), antioxidant (Ay *et al*., 2007; Nazemiyeh *et al*., 2008; Amroun *et al*., 2021), anti-inflammatory (Akkol *et al*., 2008; Amezouar *et al*., 2013; Amroun *et al*., 2021), analgesic (Akkol *et al*., 2008) and anticholinesterase properties (Zengin *et al*., 2019). The Table 4 illustrates the traditional use of *E. arborea*.

Table 4: Traditional use of *Erica arborea* L.

Traditional use	Part used	Country	References
Diuretic, urinary antiseptic and constipation	Leaves , flowers	**Turkey**	(Ay *et al*., 2007)
infections, The bowls of tobacco pipes Diuretic, urinary inflammations	Branches Roots Aerial part	**Italy**	(Cornara *et al*., 2009)
kidneys diseases and urinary stones, freckles, bedwetting	Flowers	**Algeria**	(Meddour and Meddour-Sahar, 2015 ; Eddaikra *et al*., 2019)
Urinary antiseptic, anti-inflammatory and hypotension	Flowers	**Iles Canarie**	(Darias *et al*., 2001)

MATERIALS AND METHODS

1. Materials

1.1. Plant materials

The fresh aerial parts of *Erica arborea* L (leaves and flowers) were harvested during the flowering stage in March 2018 from the mountain of Djebel of Tadergount, Bejaia, North of Algeria. The plant was identified by Prof. Laouer Hocine., a botanist in the Laboratory of Valorization of Natural Biological Resources, University Ferhat Abbas Setif 1, Algeria. A voucher specimen (015/DBEV/UFA/19) deposited in the herbarium located at the Department of Vegetal Biology and Ecology, University Setif 1, Algeria. The harvested materials were cleaned and then dried in shadow at room temperature for 15 days, then crushed and sieved through manual sieve and then separately powdered using an electric grinder. The resulting fine powders (leaves, flowers) were separately stored in tightly closed glass containers in the dark at room temperature.

1.2. Animals

Healthy, adult female and male albino mice (25-30g) were used. These animals were purchased from the Pasteur Institute (Algeria). Mice were housed in different cages under standard conditions of 12:12 h light/dark cycle and 25 ± 1°C for one week before the experiments. They were given free access to water and standard diet (*ad libitum*), and kept under standard conditions mentioned in the Animals By-Laws N° 425–2008. The experimental assays were approved by the Committee of the 'Association Algerienne des Sciences en Experimentation Animals (http://aasea.asso.dz/articles/) under law No. 88-08/1988, associated with veterinary medical activities and animal health protection (N° JORA: 004/1988)

1.3. Microorganisms strains

Reference microorganisms used in this study represent pathogenic species and were obtained from the American Type Culture Collection (ATCC). The bacteria were consisted of three Gram-negative strains: *Escherichia coli* (ATCC11303), *Pseudomonas aeruginosa* (ATCC27853) and *Salmonella gallinarum* (ATCC700623), and three Gram-positive strains: *Bacillus cereus* (ATCC10987), *Micrococcus luteus* (ATCC27141) and *Staphylococcus aureus* (ATCC25923), which were maintained in sterile nutrient agar. All the bacterial strains were subcultured from the original culture, stored at −20 °C and maintained on Muller-Hinton (MH) agar plates at 4 °C, and grown at 37 °C when required. The strains used are those recommended by quality control and compliance laboratory «Ghaouat» d'AinMelila (Algeria).

1.4. Chemicals and reagents

The used chemicals and reagents were purchased from Sigma (Germany), Fluka, Prolab and Biochem.

2. Methods

2.1. Ethnopharmacological survey

2.1.1. Study region

The study was conducted in mountain Djebel Tadergount Derguina-Bejaia and around the study district, (Figure 10) in Algeria which is located in the north of Africa (36°32'59.07" North and 05°18'35.18" East). About 14146 habitant live in this study area and the people of this region are mostly depended on the resources coming from the mountainous areas for therapeutic purpose.

Figure 10: Geographical location of study area**.**

2.1.2. Informants and collection of ethnomedicinal data

In order to identify and establish a list of plants used in traditional medicine due to their pharmacological properties, an ethnobotanical survey was conducted according to Karbab *et al*., (2020) with few modifications. In our study, information concerning traditional use of plants in the study region was collected from villagers, traditional healers, and herbalists. One hundred and seventy one informants including 99 male and 72 female of ages ranging between 30 to 65 years old were interviewed.

2.2. Extraction procedures profile

2.2.1. Fractionation of crude methanolic extracts

The *Erica arborea* hydromethanolic extracts (EAME) from different parts were prepared by using maceration technique (Merkham *et al*., 1982). Methanol was used as an extractant in this extraction using a ratio of 1:10 of plant materials to extracting. Briefly, 100 g of each grinded vegetal part (leaves, flowers) were extracted in a methanol-water mixture (850-150) at room temperature with occasional stirring during twenty-four hours. The mixture was filtered and residue was extracted with two additional 1000 mL of methanol-water (500-500) for 4h. The hydromethanolic extract was sequentially extracted with organic solvents (hexane, chloroform, ethyl acetate) in order of increasing polarity by using separating funnel (Figure 11). All the four fractions of each plant extract were dried until use.

2.2.2. Preparation of decoction extracts

Decoction extracts were obtained by mixing 30 g of dried plant material in 300 mL of boiled distilled water and then heated for 20 min at 100 °C under magnetic agitation according to method of Ghanda *et al*. (2013). After filtration by muslin cloth and centrifugation at 4000 rpm for 20 min, the filtrate was concentred in an oven at 40°C to obtain decoction extracts, then stored at 4°C untill use.

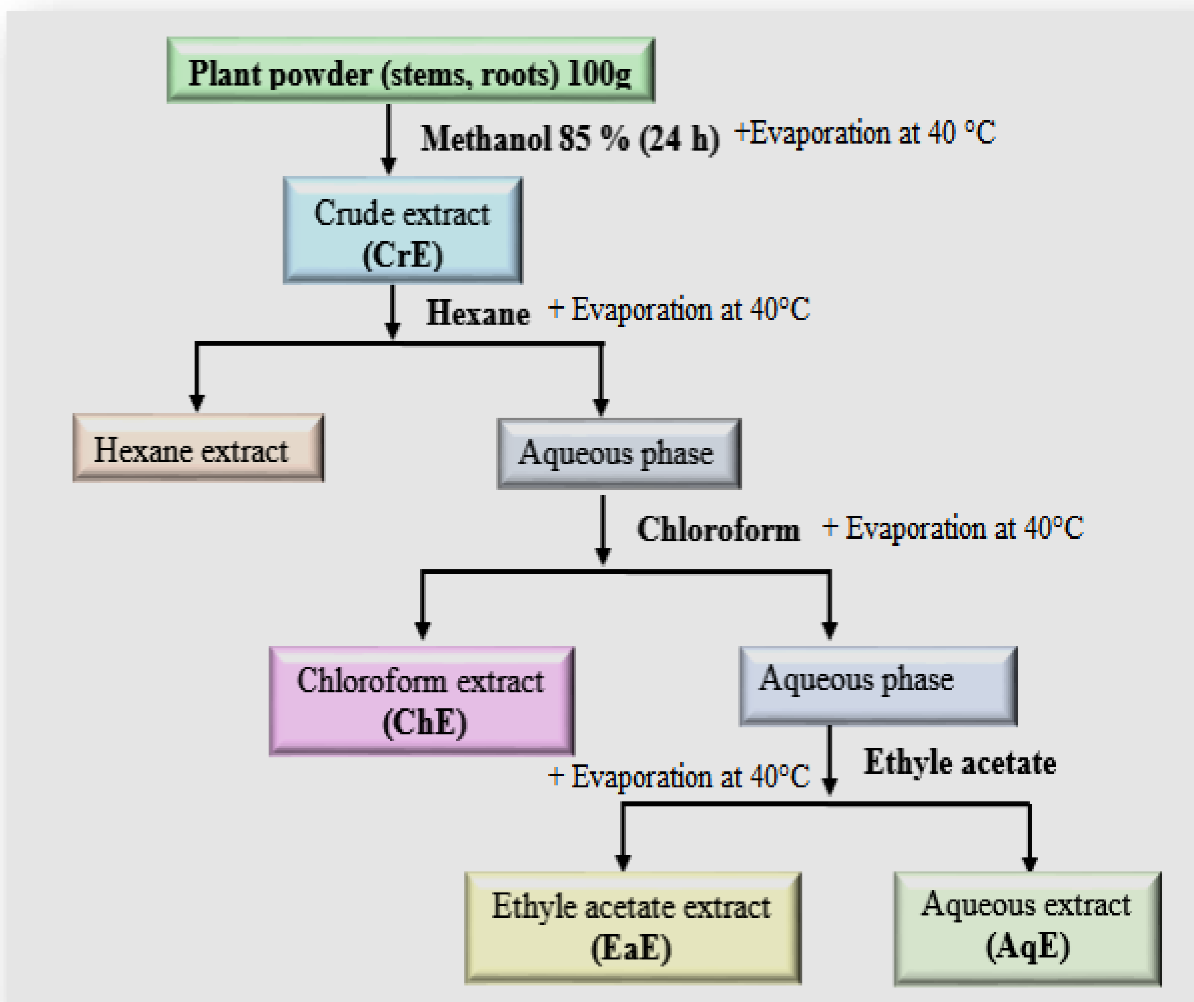

Figure 11: Sub- fractions of crude extracts of leaves and flowers.

2.3. Phytochemical investigation

2.3.1. Qualitative phytochemical screening

The extracts of *Erica arborea* were subjected to detect the presence of potential phytochemical constituents such as polyphenols, flavonoids, hydrolysable tannins, anthraquinones, quinones, anthocyanins, coumarins, alkaloids, terpenoids and saponins according to published procedure.These are qualitative analyses based on coloring and/or precipitation reactions.

2.3.1.1. ***Detection of alkaloids*****:** about 50 mg of residue is taken up in 3 ml of ethanol water (60-40 v/v). After stirring, 2 drops of Dragendorff reagent were added. The appearance of an orange-red or reddish-brown precipitate indicates a positive test (Karbab, 2020).

2.3.1.2. ***Detection of Polyphenols*****:** a few drops of 2% alcoholic of ferric chloride were added to 2 mL of the extract. The appearance of darkish blue or green color indicates the presence of polyphenols (Karbab, 2020).

2.3.1.3. ***Detection of flavonoids*****:** about 5 mL of extract were treated with few drops of concentrated HCL. After adding little quantity of magnesium turnings. The appearance of a red or orange color indicates the presence of flavonoids (Karbab, 2020).

2.3.1.4. ***Detection of hydrolysable tannins*****:** about 2 mL of extract was reacted with little sodium acetate (CH_3CO_2Na); then, a few drops of a 2% $FeCl_3$ aqueous solution were added. The reaction is positive if a blue-black color appears (Karbab, 2020).

2.3.1.5. ***Detection of free quinones*****:** a few drops of (1% in H_2O) sodium hydroxide were added to 5 mL of the extract. The appearance of yellow, red or purple colors indicated the presence of free quinones (Karbab, 2020).

2.3.1.6. Detection of anthraquinones: a few drops of (10%) ammonium hydroxide were added to 10 mL of the extract. The appearance of red ring color indicated the presence of anthraquinones (Karbab, 2020).

2.3.1.7. ***Detection of coumarins*****:** about 2 mL of alcoholic solution obtained from stock solution (dissolving 500 mg of each extract in 100 mL of ethanol) are introduced in the two test tubes. In one tube is added 0.5 mL of 10% NaOH and the test tube was heated in a water bath to boiling. After cooling, 4 mL of distilled water are added to each test tube. The reaction is positive; if the test tube in which the alkaline solution has been added is transparent compared to the control test tube. After acidifying the alkaline solution with a few drops of concentrated HCl, it loses its yellow color and becomes cloudy or precipitate forms (Karbab, 2020).

2.3.1.8. ***Detection of terpenanoids*****:** about 2.5 mL of extract was added 1 mL of chloroform. After homogenization, 1.5 mL of concentrated H_2SO_4 was added to the mixture. The formation of a brownish red color at the interface indicates the presence of these compounds (Karbab, 2020).

2.3.1.9. *Detection of anthocyanins*: the presence of anthocyanins was indicated by a red coloration which was emphasized by the addition of dilute HCl and turned to blue-violet-greenish coloration by the addition of ammonia (Karbab, 2020).

2.3.1.10. *Detection of saponins*: about 5 mL of the extract was well shaken with 10 mL of distilled water for 2 min. The appearance of foam that persists after 15 minutes confirms the presence of saponins (Karbab, 2020).

2.3.2. Quantitative phytochemical analysis

2.3.2.1. Determination of total polyphenols

The total phenolic contents in the extracts of *E. arborea* from different parts were determined by the Folin-Ciocalteau's method (Karbab *et al.*, 2019). A calibration curve was prepared using gallic acid as standard (10–160 µg/mL). Briefly, an aliquot of standard/extracts solution (200 µL) was mixed with 1 mL Folin-Ciocalteau's reagent (diluted ten-fold with water). After 4 min of incubation, 800 µL of aqueous sodium carbonate (7.5%) was added. The absorbance was measured at 765 nm after 2 h of incubation at a dark room temperature. The total phenolic contents in the extracts were expressed as mg gallic acid equivalents per microgram of dry extract (GAE)/g of dry weight (DW).

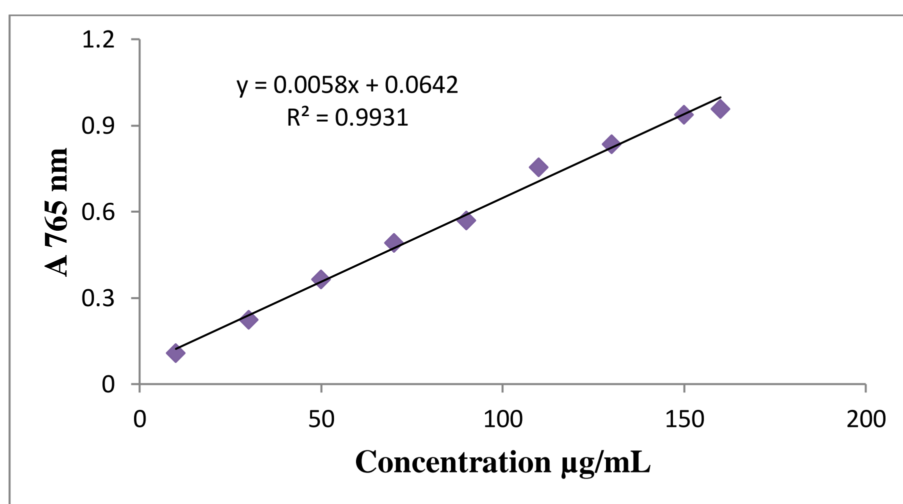

Figure 12: Standard curve of gallic acid for determination of total polyphenols in *E. arborea* extracts. Each value represent mean ± SD (n=3).

2.3.2.2. Determination of total flavonoids

The total flavonoid contents were determined by the aluminium chloride ($AlCl_3$) method (Karbab *et al.*, 2019). A calibration curve was constructed using quercetin (2.5 - 40 µg/mL). 1mL of standard/extracts was mixed with 1 mL of aluminium chloride reagent (2%). After 10 min of

reaction, the absorbance was measured at 430 nm. The concentration of flavonoids was determined by reference to a calibration curve using quercetin (2.5 - 40 µg/mL) prepared in the same way as the extract. The content of flavonoids was expressed as µg of quercetin equivalent per microgram of dry weight (µg QE/mg DW). All determinations were carried out in triplicate (*n* = 3).

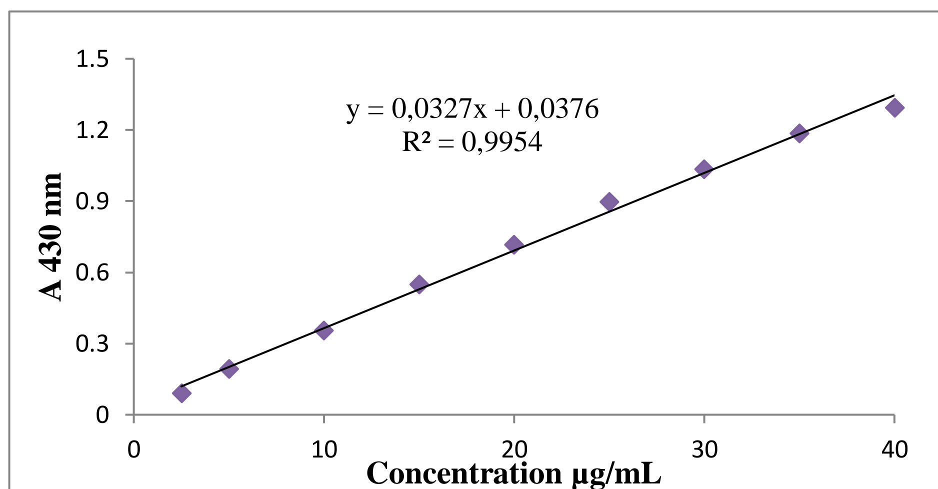

Figure 13: Standard curve of quercetin for determination of total flavonoids in *E. arborea* L extracts. Each value represent mean± SD (n=3).

2.3.2.3. Determination of condensed tannins

Determination of condensed tannins was performed by the vanillin essay with HCl. This method depends on the reaction of vanillin with the terminal flavonoid group of condensed tannins (TC) and the formation of red complexes (Ali-rachedi *et al*., 2018). A volume of 1 mL of each extract was added to 1.5 mL of the 4% vanillin/methanol solution, and then mixed vigorously. Then a volume of 750 µL of concentrated hydrochloric acid (HCl) was added. The mixture was allowed to stand for 20 min at 20 ◦C in the dark. The absorbance was measured at 500 nm against a blank. Different concentrations (100 to 500 µg/mL) were prepared from a stock solution of catechin and were used to draw the calibration curve. The final results were expressed as mg catechin equivalent (CE) per g of dry weight (DW). Each experiment was repeated at least three times.

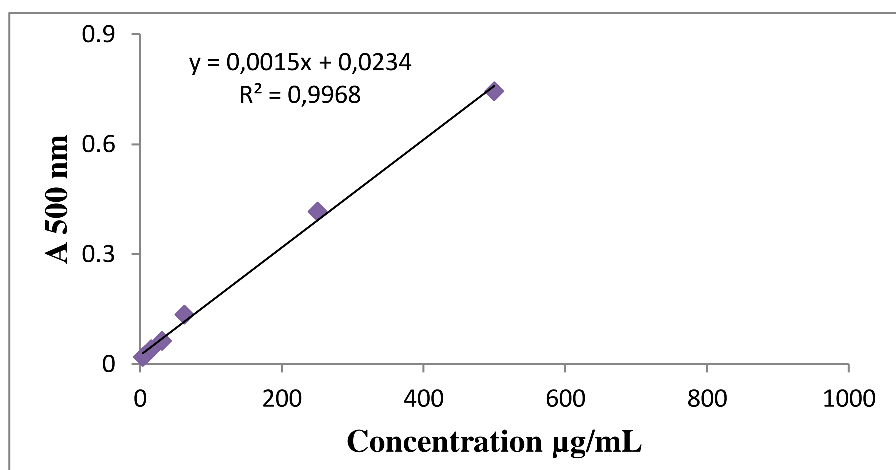

Figure 14: Standard curve of catechin for determination of condensed tannins in E. *arborea* L extracts. Each value represent mean± SD (n=3).

2.3.3. HPLC-MS analysis

Using Elute UHPLC coupled to a Bruker impact II QTOF-MS detector. Chromatographic separation was performed using a Bruker solo 2.0-C18 UHPLC column (100 mm x 2.1 mm x 2.0 µm). Three µL of the sample were injected. The flow rate was 0.51 mL / min and a column temperature was 40 °C. A linear gradient was performed using binary solvents (A composed of 0.05% formic acid in water and B composed of 100% acetonitrile).The gradient started at an initial ratio of 95:5% (A:B) and was ramped to 20:80% over 27 min. The gradient was ramped linearly to 5:95% in 2 min, then elution was continued with 5% B until 35 min.

A Bruker Daltonik (Bremen, Germany) impact II ESI-Q-TOF System was used to screen compounds of interest, using direct injection. This instrument was operated using the Ion Source Apollo II ion Funnel electrospray source. The capillary voltage was 2500 V, the nebulizer gas was 2.0 bar, the dry gas (nitrogen) flow was 8 L/min and the dry temperature was 200º°C. The mass accuracy was < 1 ppm; the mass resolution was 50000 FSR (Full Sensitivity Resolution) and the TOF repetition rate was up to 20 kHz. We identified compounds in the sample by comparison of their mass spectra and retention time with those of commercially available standards of available standards. All the reagents and standards used were LC/MS grade (Al-Jaber *et al*., 2020).

2.4. Pharmacological assays

2.4.1. In vitro antioxidant activity

2.4.1.1. DPPH antioxidant assay

To determine the antioxidant activity of methanolic extracts of various parts of *E. arborea*, the DPPH assay was performed to estimate the scavenging ability of various extracts by quenching DPPH (Charef *et al.*, 2015). The assay was performed in triplicate, and the mean absorbance was calculated and noted at 517 nm. Briefly, freshly prepared DPPH solution (0,4mM) was prepared in methanol, stored in an amber color bottle. All the *E. arborea* were dissolved in methanol and make various concentrations of an extract as well as butylatedhydroxytoluene (BHT), which was used positive control by applying serial dilution method. Add 1.25 mL of DPPH solution to 50 µL of each serial dilution of extract and butylatedhydroxytoluene. Additionally, 1.25 mL of butylatedhydroxytoluene BHT solution is mixed with 50 µL of methanol as control without extract or butylatedhydroxytoluene. The mixture was mixed well and incubated in darkness for 30 min. The percent of inhibition of DPPH radical was calculated using the formula:

$$\text{Radical scavenging activity } \mathbf{(\%) = [(A_{blank} - A_{test})/A_{blank}]*100}$$

Where $\mathbf{A_{blank}}$: the absorbance of the solution except the tested sample and $\mathbf{A_{test}}$: the absorbance of the tested extract or standard.

2.4.1.2. Reducing power assay

The reducing power assay was performed to estimate the ability of various extracts to reduce Fe^{+3} $(CN^-)_6$ to Fe^{+2} $(CN^-)_6$ (Bouaziz *et al.*, 2015), with some modifications. According to this procedure, an aliquot of 400 µL of extract was mixed with an identical volume of both phosphate buffer (0.2 M, PH= 6.6) and potassium ferricyanide (1%). This mixture was then incubated for 20 min at 50°C in a water bath. The reaction was terminated by adding 400 µL of trichloroacetic acid (TCA) (10%), and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (400 µL) was added to distilled water (400 µL) and 80 µl of 0.1% ferric acid. The color intensity of the mixture was measured at 700 nm after 10min of incubation. In this context, a high absorbance of the solution means a high reducing power.

2.4.2. Antibacterial activity

2.4.2.1. Qualitative antibacterial activity by agar disk-diffusion method

For determined the sensitivity or resistance of the bacteria, agar disk-diffusion method was employed (Yaici *et al.*, 2019). Bacterial strains were cultured at 37 °C for 24 h in nutrient broth (BN). The different bacterial strains were subcultured to obtain a young culture, which was used to prepare the bacterial inoculum with an optical density of McFarlend 0.5 (10^8 Colony Forming Units/mL) in sterile sodium chloride (0.9 %). Dried plates (90 mm of diameter) were inoculated by the bacterial inoculums which were prepared previously using a sterile cotton swab. The extracts were weighed (100 mg each) and dissolved in 1 mL of dimethylsulphoxide (DMSO) respectively, making up the final testing concentration of each extract as 100 mg/mL. All test samples and their respective diluted concentrations were then prepared. Gentamicin (0.12 mg) was used as a control for the disk diffusion assay. Sterilized discs of 6 mm in diameter (Wathman N°.3) were prepared. The discs were then deposited on the surface of the Muller Hinton gelose of the dried plates with 10 µL of different concentrations of extracts (12.5, 25, 50 and 100 mg/mL). These plates were incubated for 24 hours at 37 °C and all plates were examined for any zones of growth inhibition, and the diameter of these zones were measured in millimeters using a caliper. All the tests were repeated twice.

2.4.2.2. Quantitative antibacterial activity by broth microdilution method

A widely accepted sensitive serial dilution microplate method (Elisha *et al.*, 2017) was used to determine the minimum inhibitory concentration (MIC) of *E. arborea* extracts against six bacterial strains in triplicate with some modifications. Bacterial cultures grown overnight were adjusted in sterile nutrient broth to a turbidity of 0.5 on the McFarland (10^8 Colony Forming Units/mL) as an equivalent to the density of (0.08 - 0.10) at 625 nm. The tested dried extracts were dissolved in DMSO to a concentration of 100 mg/mL and 50 µL was added to the first well of a 96-well microplate and serially transferred from 1th well to the 12th one, whereas, the last volume of 50 µL was discarded. Bacterial suspensions (100 µL) were added to each well. Gentamicin was used as positive control and DMSO was used as a solvent control. The microplate was covered and incubated overnight at 37 °C. A sterility control well and a growth control well were also studied for each strain. Each experiment was repeated at least three times. After 24 h of incubation, the microplates were subjected to spectrophotometry and the absorbances were measured at 625 nm. The activity was defined as the lowest absorbance that led to growth inhibition. The determination of minimum bactericidal concentration (MBC) is also calculated from MIC data.

2.4.3. Anti-inflammatory activity

2.4.3.1. In vivo topical anti-inflammatory effect

2.4.3.1.1. Xylene-induced ear edema

The anti-inflammatory activity of the extracts from *E. arborea* was investigated in xylene-induced ear edema in mice (Karbab *et al.*, 2020). Mice were divided into five groups of 6 animals each (*n* = 6). Group n°=I (positive control), received topically with indomethacin used as a standards drugs (0.5 mg/ear), group n°=II (negative control), received topically xylene. Groups III-V received topically different extracts, respectively. First, the edema was topically induced in each mouse using 30 µL/ear of xylene. Simultaneously, 30 µL of water solution containing 0.5 mg of different extracts and indomethacin were topically applied at the same place of the ear; mice of the control group topically received only the xylene. The thickness of the ear measured with a digital calliper before and 2 h after the xylene application. The inhibition percentage of ear edema was computed according to the formula:

$$\text{Inhibition percentage (\%)} = 100 \times (\Delta T - \Delta E)/\Delta T$$

Where ΔT is the difference of ear edema thickness in the negative control, ΔE is the difference of ear edema thickness in the hydromethanolic extracts or positive control.

2.4.3.1.2. Croton oil-induced ear edema

The anti-inflammatory activity of extracts was evaluated using the croton oil-induced ear edema method in mouse model (Karbab *et al.*, 2019). Mice were randomly divided into five groups of six mice each. Group I (positive control), received topically indomethacin used as a standard drug, group II (negative control), received topically croton-oil solution, whereas groups III–V received topically the different extracts, respectively. First, 15 µL of acetone-water solution (1:1, *v/v*) containing 80 µg of croton oil was topically applied at the inner surface of the right ear of each mouse. Simultaneously, 15 µL of acetone-water solution (1:1, *v/v*) containing 0.5 mg of different extracts and indomethacin were topically applied at the same place of the ear; mice of the control group topically received only the solution of croton oil. The thickness of the ear was measured by means of a digital caliper before treatment and 6 h after induction of inflammation. The difference in thickness before and after the application of croton oil was calculated. The inhibition percentage of ear edema was computed as in the equation of xylene-induced ear edema activity.

2.4.3.2. In vivo oral anti-inflammatory effect

2.4.3.2.1. Xylene-induced ear edema

The anti-inflammatory activity of the extracts from *E. arborea* was investigated in xylene-induced ear edema in mice (Karbab *et al*., 2020). Mice were divided into eleven groups of 6 animals each (*n* = 6). Group n°=1 (positive control): received orally with indomethacin used as a standards drugs (50 mg/Kg), group n°=2: (negative control), received orally distilled water. Groups III-V received orally hydro-methanol extract from stem (100, 300, and 600 mg/kg in 0.5 mL H_2O, respectively), groups VI-VIII received orally hydro-methanol extract from flower and groups VIII-XI received orally hydro-methanol extract from leaves by the same doses of groups III-V, respectively. After 60 min of treatment, edema was then topically induced in each mouse using topically 30 µL/ear of xylene. The thickness of the ear measured with a digital caliper before and 2 h after the xylene application. The inhibition percentage of ear edema was computed as in the equation of xylene-induced ear edema activity.

2.4.3.2.2 .Croton oil-induced ear edema

The anti-inflammatory activity of the extracts from *E. arborea* was investigated in croton oil-induced ear edema in mice (Karbab, 2020). Mice were randomized into eleven groups of 6 mice each. Group n°=I (positive control), received orally with indomethacin used as a standards drugs (50 mg/Kg), group n°=II (negative control), received orally distilled water. Groups III-V received orally hydro-methanol extract from stem (100, 300, and 600 mg/kg in 0.5 mL H_2O, respectively), groups VI-VIII received orally hydro-methanol extract from flower and groups VIII-XI received orally hydro-methanol extract from leaf by the same doses of groups III-V, respectively. The induction of inflammation with 15 µL croton oil solution (80 µg in 50% water-acetone v/v) was locally applied in the inner surface of the right ear of mice after 1 h of administration. The thickness of the ear was measured by means of a digital caliper before treatment and 6 h after the induction of inflammation. The inhibition percentage of ear edema was computed as in the equation of xylene-induced ear edema activity.

2.4.4. Analgesic activity

Writhing study was performed to investigate the analgesic effect of hydromethanolic extracts against acetic acid-induced abdominal constriction (Karbab *et al.*, 2020). In this protocol, female mice were divided into eleven groups of five animals each (n = 5). Group I: (positive control), treated orally with aspirin, used as standard drug (100 mg/Kg). Group II: (negative control), treated orally with distilled water. Groups III-V received orally hydro-methanol extract from stem (100, 250, and 500 mg/kg in 0.5 mL H_2O, respectively), groups VI-VIII received orally hydro-methanol extract from flower and groups VIII-XI received orally hydro-methanol extract from leaf by the same doses of groups III-V, respectively. After 60 min, writhes was induced in mice through intra-peritoneal injection with 0.1% (v/v) acetic acid. The number of abdominal contractions was counted over a period of 30 min, after 5 min of injection. The percentage inhibition of writhing reflex was calculated using the formula: Inhibition (%) = 100 x $(C_n – C_t)/C_n$, where C_n = Mean of contractions' count in animals in the negative control, and C_t = Mean of contractions' count in animals treated with different concentrations of hydromethanolic extracts and aspirin.

2.4.5. Anti-urolithic activity

2.4.5.1. Nucleation assay

Effect of extracts on calcium oxalate crystal formation was carried out by nucleation assay according to a previously described method of Salem *et al.* (2020). Calcium chloride (0.5 g/L) and sodium oxalate solution (0.75 g/L) were prepared in Tris-HCl (5 g/L) buffer (pH 6.5). Dilutions of extract ranging from (62.5-500) µg/mL were prepared in buffer. 100 µL of each extract concentration was mixed with 950 µL calcium chloride solution followed by the addition of 950 µL sodium oxalate solutions. Final mixtures were incubated for 30 min at 37ºC. The absorbance of the mixtures was then measured at 620 nm wavelength. Percent inhibition of nucleation by extracts was calculated using the under mentioned formula and compared to that calculated for the standard allopurinol.

$$\mathbf{I\ (\%) = 100 \times (1 - A_{test}/A_{control})}$$

Where $A_{control}$ = absorbance of the negative control, and A_{test} = absorbance of the hydromethanolic extracts and allopurinol.

2.4.5.2. Aggregation assay

The calcium oxalate crystal aggregation was determined by aggregation assay described by Salem *et al.* (2020). The calcium oxalate crystals were prepared by mixing together calcium chloride and

sodium oxalate (50 mmoL/ each). The mixed solution were heated in a water bath for 1 h to 60 ºC and then incubated overnight at 37ºC. After drying, CaOx crystals were used at a final concentration of 0.8 mg/mL, buffered with Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. 200 µL of extract (62.5-500) µg/mL of extract were added to 600 µL calcium oxalate solution, vortexed and then incubated at 37ºC for 30 min. The absorbance of the final mixtures was then read at 620 nm wavelength and percent inhibition of aggregation was then calculated as described for nucleation assay.

2.4.6. Acute oral toxicity of *E. arborea*

Forty-two albino mice were designed for acute oral toxicity study of the methanolic extract, using the guidelines internationally accepted (425) (OECD, 2008), weighing between 20-27g, and divided into seven groups (n = 6). Each group of six mice (3 males and 3 females) in the I-VII groups were subjected to fasting for 12 h before the experiment period and received orally two doses of EAME (leaves and flowers) of 2000 and 5000 mg/kg (in 1 mL H_20), respectively, whereas mice in the VIII group (negative control) were given distilled water.

2.5. Statistical analysis

Determinations were conducted in triplicate and results are expressed as the mean ± standard deviation (SD).We employed Graph Pad Prism-6 (Graph Pad Software, San Diego, California USA, http://www.graphpad.com) to analyze data obtained from this investigation. Data were subjected to one-way analysis of variance (ANOVA), for significance. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

1. Ethnobotanical and ethnopharmacological survey

The plant material to be investigated can be selected based on some specific traditional ethnomedicinal uses. The traditional medicine constitutes an important and often underestimated source of health care for multiples diseases. However little is known about their ethnomedical knowledge and their practices in Northern Africa (Taïbi *et al.*, 2020). In addition, ethnopharmacology can be also defined as the scientific interdisciplinary of the active agents exploration, that are traditionally employed (Süntar, 2019) and preserved the ancestral knowledge of populations (Salhi *et al.*, 2019). Ethnobotanical information is shown in Table 5. A total of 20 species of medicinal plants divided between 18 genus and 15 families are mentioned. Data from the ethnopharmacological study on the use of medicinal plants in the treatment of different diseases revealed from traditional healers in the study area. All of the 171 participants who randomly selected in the study (99 men and 72 women) were locals. Evaluation of our investigation revealed that *Erica arborea* was the most common plant utilized by people (48 mentions).

1.1. Medicinal plants used

The present study show a diversity of medicinal plants identified and used in folk medicine in the north of Algeria, which are presented in table 6. Among these 20 spontaneous species, three of them that are most mentioned *Erica arborea, Inula visqusa* and *Pistacia lentiscus* with percentages of 28.04 %, 16.37 % and 15.20 %, respectively. The distributions of the percentages of traditional plant families use are shown in Figure 15.

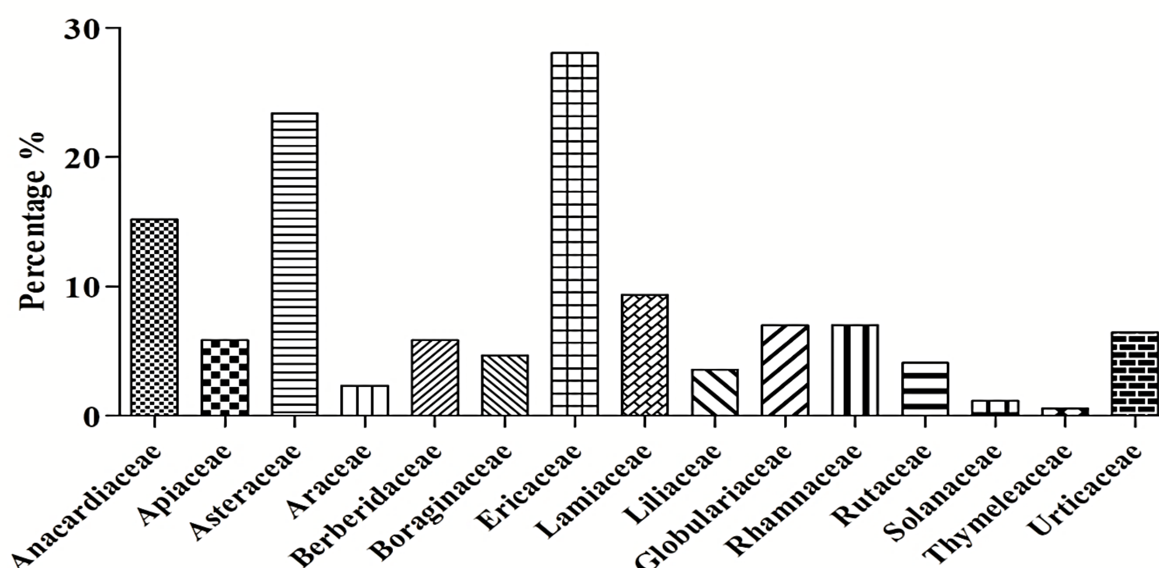

Figure 15: Distributions of traditional plant family use rates in the study area.

Table 5: Ethnobotanical study of some medicinal plants used in traditional medicine (Tadergount, Derguina-Bejaia, North of Algeria).

Medicinal plants/family	Local names	Parts used/ method of administration	Traditional usage	Total mentions
Pistacia lentiscus L. **(Anacardiaceae)**	Thidekt	L/Ext and Int	Burn, canker sores, hair dandruff, and gastric diseases	**26**
Thapsia garganica L. **(Apiaceae)**	Darias	S/Ext	Back pain	**10**
Inula visqusa L. *Artemisia absinthium* L. *Artimisia herba-alba* L. **(Asteraceae)**	Amagramane Chadjrtmariem Chih	L/Ext AP/Int R, L/Int	Wounds and back pain hemorrhoids diarrhea and stomach aches	**28** **04** **08**
Arum italicum L. **(Araceae)**	Avequk	AP/Ext	Hemorrhoids and dermatological diseases	**04**
Berberis vulgaris L. **(Berberidaceae)**	Barazetem	R/Ext and Int	Gastrointestinal and genecology diseases	**10**
Cynoglossum creticum L. **(Boraginaceae)**	Thijrarehiyine	L/ Ext	Wounds, burns, and stomach aches	**08**
Erica arborea L. **(Ericaceae)**	Bouhadad, khlenj	L, F and AP/ Ext and Int	Eczema, kidney stones, urinary and gastric diseases, inflammation and microbial infections and snakebites.	**48**
Lavandula stoechas L. *Ajuga iva* L. **(Lamiaceae)**	Amzir Chandekora	L, F/Int L/Ext and Int	Diabetes and gastric diseases, hypertension, stomach ache, and headache	**12** **04**
Asphodelus microcarpus L. **(Liliaceae)**	Avarwak	R/ Ext and Int	Yeast infection, and otitis	**06**
Globularia alypum L **(Globulariaceae)**	Marziza	R/ Ext	Burn healing, cream	**12**
Rhamnus alaternus L. **(Rhamnaceae)**	Imliles	L/Int	Anemia	**12**
Ruta angustifolia L. **(Rutaceae)**	Fijla, Awarmi	L/Int	Gastric diseases	**07**
Hyoscyamus niger L. **(Solanaceae)**	Vounarjouf	L/Ext	Eczema and influenza	**02**
Daphne gnidium L. **(Thymeleaceae)**	Alzaz	L/Ext	Yeast infection, arthrosis	**01**
Urtica dioica L. **(Urticaceae)**	Azetouf	AP/Ext and Int	Rheumatism, hair loss, and hemorrhoids	**11**

AP: Aerial part, L: Leaves, R: Roots, F: Flowers, S: Stems, Ext: External, Int: Internal

1.2. Plant parts used

The plant parts used by informants for treatment include leaves, flowers and whole aerial parts (Table 6). *E. arborea* plant contributes to primary health care for the treatment of kidney stones diseases, gastrointestinal pain, urinary infections and inflammatory skin diseases. Medicinal uses of the *Erica arborea* were divided into 5 different categories, of which the uses for kidney stone and inflammation diseases (48%), urinary infections (14%), gastrointestinal (20%), eczema (4%), snakebites (4%) and 10% for other diseases. Internal application is the most route used for all respondents with a percentage of 84% followed by external with 16% for plant parts.

Table 6: Distribution of the different parts of *E. arborea* and route of application

Parts used	Route of use	Mode of preparation/treatment
Leaves	Internal	Infusion/ urinary antiseptic, gastric problems, diuretic, inflammation, kidney stones and microbial infections
	External	Cataplasm/ microbial infections, betting of serpents and insects and kidney stone diseases
Flowers	Internal	Infusion/ urinary antiseptic, gastric problems, microbial infections,diuretic, prostate cystis and kidney stones diseases
	External	Cream/ treatment of freckles, microbial infections and kidney stone diseases
Aerial parts	Internal	Infusion, decoction/ urinary antiseptic, diuretic, astringent and laxative, kidney stone diseases, inflammation and microbial infections

2. Extraction yields

There are many steps to obtain phytochemicals from plant such as milling, grinding, homogenization, and extraction. Due to the structural diversity and complexity of phenolic compound in plants (Bujor, 2018). The extraction is the most main step for recovering and isolating of phytochemicals from plants materials (Do *et al.*, 2014). Today, a number of different extraction techniques are used (Vujanović *et al.*, 2019). The most common liquid/liquid and solid/liquid extractions are frequently employed to separate phenolic compounds (Bujor, 2018).

The extraction procedures and solvents are responsibles for dissolving the endogenous compounds of the plants (Aryal *et al*., 2019).

In this study, solvent extraction procedures applied to *E. arborea* include maceration with fractionation and decoction. In the first one, an extraction with hydromethanolic mixture methanol water was initially performed to obtain the crude extract, which containing the total secondary metabolites. In the following step is the fractionation of crude extract; a series of solvents by varying polarities (hexane, chloroform, ethyl acetate) was employed to separate the crude methanol extract according to their degree of solubility in organic extraction solvents. Thereby, 12 extracts were obtained successively. The second method is water utilization as an extractant in decoction protocol, which was allowed to get 3 extracts. The yield of each extract is shown in Table 7.

Table7: Yield, color and consistency of *E. arborea*. L extracts.

Extracts	Extraction Yield (%)		Color and Consistency	
	LE	**FE**	**LE**	**FE**
DecE	6.49	4.28	Dark brownish mirrored powder	Dark brownish mirrored powder
CrE	21.21	21.28	Dark brownish mirrored powder	Dark brownish mirrored powder
ChE	1.36	1.2	Dark greenish mate powder	Clair greenish mate powder
EaE	6.84	5.32	Clair orangish mirrored powder	Clair orangish mirrored powder
AqE	15.81	12.20	Cark brownish mirrored powder	Dark brownish mirrored powder

DecE: decoction extract; CrE: crud extract; ChE: chloroform extract; EaE: ethyl acetate extract; AqE: aqueous extract LE: leaves extracts; FE: flowers extracts.

Compared with other modern techniques of extraction, maceration is desirable for their advantages and decoctions was the most common method traditionally employed and powerful method for isolating of the bioactive metabolites of medicinal plants (Sharafatmandrad and Mashizi, 2020). The presence of a hydroxyl group in polyphenols makes them relatively hydrophilic in water (Bujor, 2018; Aryal *et al*., 2019).

Other extracting solvent can also affect the quantity of the active compounds in the extract, where non-polar compound dissolve in non-polar solvent such as lipophilic phenols and polar compounds will dissolve in polar solvent such as hydrophilic phenols (Do *et al.*, 2014; Masoko, 2017; Medini *et al.*, 2014; Savitri *et al.*, 2019).

According to the results of Table 7, the variability of extraction yield depends on the plant part, extraction processes, as well as the solvent used. Do *et al.* (2014) reported the presence of interfering substances is an indicator, who can also affect the extraction yield. The highest yield was noted with crude extract (CrE) in all used parts compared to other extraction by various solvents. The yields decreased in the following order: CrE> AqE> EaE> DecE> ChE of *E. arborea;* which ranged from 21.28 to 1.2 % for flowers and 21.21 to 1.36 % for leaves.

Auxiliary, it was observed that the CrE of flowers and leaves produced an almost similar maximum yield of phytochemicals about 21.28 and 21.21%, respectively. Although, chloroform extract (ChE) of leaves and flowers displayed a lower yields with range from 1.2 to 1.36%. Color and consistency of extract varied also according to the extraction solvents, a mirrored powdery are recorded for the decoction (DecE), CrE, ethyle acetate (EaE) and aqueous extract (AqE), while the ChE of leaves and flowers has a matte powdery appearance. The extraction yield of CrE of leaves is larger to those obtained by the previous scientific report of Luís *et al.* (2011); this could be attributed to the extraction procedure employed.

3. Phytochemical analysis

3.1. Phytochemical screening

In the context to gain preliminary knowledge on the nature of metabolites presents in the used materials, systematic phytochemical investigation is accomplished. The preliminary results from the phytochemical study of the studied extracts are shown in Table 8. The results showed that all the fractions of leaves (L) and flowers (F) contained comparable phytochemical substances such as polyphenols, quinones and terpenoids. Whereas, anthocyanins, coumarins, and alkaloids substances were found to be absents in all extracts. Flavonoids were present in CrE and EaE of (L) and (F) with strong extent. Further, the presence of large amount of hydrolysable tannins was revealed in CrE, decoction (DecE) and aqueous (AqE) extracts of (L) and (F).

Table 8: Phytochemical screening of different extracts from *E. arborea* L. parts.

Phytochemicals		Extracts				
		CrE	DecE	ChE	EaE	AqE
Polyphenols	LE	+++	+++	+	+++	++
	FE	+++	+++	+	++	++
Flavonoids	LE	++	-	-	+++	++
	FE	++	-	-	+++	-
Hydrolysable tannins	LE	+++	+++	-	-	+++
	FE	+++	+++	-	-	+++
Saponins	LE	-	++	+++	-	++
	FE	-	++	+++	-	+
Anthraquinones	LE	+	+++	-	+++	+
	FE	+	+++	-	+++	+
Quinones	LE	++	+	+	++	++
	FE	++	++	+	++	++
Coumarins	LE	-	-	-	-	-
	FE	-	-	-	-	-
Anthocyananins	LE	-	-	-	-	-
	FE	-	-	-	-	-
Terpenoids	LE	++	++	++	++	+
	FE	++	++	++	++	+
Alkaloids	LE	-	-	-	-	-
	FE	-	-	-	-	-

+ = less presence, ++ =middle presence, +++ = fort presence, - =absence. DecE: decoction extract; CrE: crude extract; ChE: chloroform extract; EaE: ethyl acetate extract; AqE: Aqueous extract; LE: leaves extracts; FE: flowers extracts.

Some extracts showed the presence of small amounts of saponins such as DecE, and AqE. The chloroform extract (ChE) showed the presence of saponins with strong extent. The anthraquinones were found in all extracts, except for ChE in which the absence of anthraquinones was detected. Polyphenols, hydrolysable tannins, terpenoids, quinones, and anthraquinones detected in *E. arborea* extracts were in agreement with those obtained by Amezouar *et al.* (2013) from the leaves methanolic extract of *E. arborea* in which the presence of saponins was also reported.

According to the several researchers, polyphenols, flavonoids, tannins, saponins, quinones, anthraquinones, and terpenoids are widely known for their essential medicinal properties such as antioxidant, antibacterial, anti-inflammatory, analgesic and antiurolithiatic activities (Wu *et al.*, 2020; Hossain *et al.*, 2021 ; Berillo *et al.*, 2022).

These molecules could be responsible for the diverse obtained biological activities. There are several factors affected nature of phytochemicals extracted from plants include extraction method, size of materials particles, the ratio of vegetal plant, solvent used and polarity of solvent (Dewi *et al*., 2020).

3.2. Total phenolic, flavonoids and condensed tannins content

The amounts of total phenolic (TPC), total flavonoids (TFC), and condensed tannins (TC) were showed in tested extracts and the results are given in Table 9.The content of phenolic, flavonoids and condensed tannins was largely influenced by extraction method, extraction solvents and its polarity, as well as by the vegetal parts.

Table 9: Total phenolic, flavonoids and condensed tannins contents of leaves and flowers from *E. arborea*. The data represents the mean ± standard deviation (SD) of three determinants.

Extracts		TPC (µg GAE/mg extract)	TFC (µg QE/mg extract)	TC (µg CE/mg extract)
DecE	LE	318.48 ± 1.95	35.59 ± 0.30	167.37 ± 1.18
	FE	417.10 ± 0.97	17.23± 0.09	273.87 ± 1.41
CrE	LE	591.58 ± 0.97	51.12 ± 1.42	337.53 ± 1.88
	FE	416.07 ± 1.46	25.25 ± 1.68	212.36 ± 0.23
ChE	LE	109.24 ± 0.49	18.53 ± 0.32	30.60 ± 1.41
	FE	80.45 ± 0.24	14.58 ± 1.99	14.77 ± 0.23
EaE	LE	944.55 ± 1.95	67.15 ± 0.04	173.53 ± 0.47
	FE	649.38 ± 1.95	65.31 ± 0.56	267.12 ± 1.06
AqE	LE	489.86 ± 1.46	9.37 ± 0.04	262.62 ± 0.12
	FE	481.24 ± 1.95	6.02 ± 0.11	280.37 ± 1.65

DecE: decoction extract; CrE: crude extract; ChE: chloroform extract; EaE: ethyl acetate extract; AqE: aqueous extract; LE: leaves extracts; FE: flowers extracts.

The largest amount of phenolic was obtained via leaves (LE), followed by flowers extracts (FE), with the exception in DecE. Firstly, in CrE, EaE and AqE extracts the highest level of polyphenols compounds were recorded in LE followed by FE. Secondly for the DecE, great values were showed in FE then LE. Thus, in all used parts, the extraction solvents had effect on TPC and ranged from 80.45 GAE/mg extract for ChE of flowers to 944.55 µg GAE/mg extract for EaE of leaves. The TPC decrease in the following order: EaE> CrE> AqE> DecE> ChE for LE and EaE> AqE> DecE >CrE

> ChE for FE. Furthermore, the content of flavonoids varying from 6.02 µg QE/mg extract for AqE of flowers to 67.15 for EaE of leaves. The largest amount of flavonoids was obtained via LE followed by FE. The TFC decrease in the following order: EaE> CrE> DecE > ChE> AqE for FE and EaE > CrE> Dec> ChE> AqE for LE.

The distribution of condensed tannins across all the used parts using different solvents exhibited also a very great difference and their content ranged from 14.77 CE/mg extract for ChE of flowers to 337.53 (µg CE/mg extract) for CrE of leaves. The TC decrease in the following order CrE>AqE>EaE>Dec>ChE for LE, AqE> CrE>EaE>Dec>ChE for FE. Some authors investigated the phenolic content of *E. arborea* in several parts of the shrub (Luis *et al*., 2011). Moreover, in methanolic leaves extract of *E. arborea*, our results disaccord with those reported by previous studies in the literature carried out by Marquez-Garcia *et al*. (2009) and Amezouar *et al.* (2013) on the alcoholic extract of leaves of same plant collected from Spain (78.49 mg GAE/g dry weight) and Morocco (81.87 mg GAE/g dry weight), respectively.

Furthermore, the methanolic *E. arborea* leaves extract, which present the concentration of total phenolic compounds, amounting to 591.58 ± 0.97(mg GAE/g dry weight), is highest than that previously reported in Algerian samples (179.6 ± 0.97 mg GAE/g) in methanolic extract (Guendouze-Boucheffa *et al*., 2015) and highest also than that reported in Turkish samples (145 mg pyrogallol equivalents /g) (Ay *et al*., 2007). The values of phenolic content varied in this current study, either compared to those in the literature in harvested areal part flowering testing for *E. arborea* from Algeria sample or in pyrogallol equivalents for *E. arborea* from Turkey sample. Suna *et al*. (2018) studied the methanol extract of dried leaves and found value at 749.48 ± 34.46 mg gallic acid equivalent/g extract) of total phenolic content and this respective amount is higher than that found in crude extract of LE (591.58 ± 0.97mg GAE/g of dry weight).

In general, LE has the highest amount of phenolic compounds. This result is similar to those obtained by Luís *et al*. (2011). Luis *et al.* (2011) examined different parts of methanol extracts from *E. arborea* such as leaves and flowers for their phenolic contents with178.1 (mg gallic acid equivalent/g extract) for flowers. This value obtained by this study was lower to that of present study. The EaE of *E. arborea* was found to have high content of total phenols especially in flowers extracts. This result is in a good agreement with the literature (Ay *et al*., 2007; Köroğlu *et al*., 2018) for aerial part of *E. arborea* Additionally, Köroğlu *et al*. (2018), conducted study of several Ericaceae species of Turkish, and concluded that their richness on phenolic compounds and the

ethyl acetate is one of the best solvents for their extraction (Köroğlu *et al.*, 2018). Maximum total phenolic content found in ethyl acetate extracts of aerial part of *E. arborea* was 315.52±3.81 (µg pyrocatechol equivalents/mg extract) and 875.5 (mg gallic acid equivalent/g extract) in previous studies of Ay *et al.*(2007) and Köroğlu *et al.* (2018), respectively.

It was observed the effect of ethyl acetate on TFC is similar to that on TPC. Polar extract showed more flavonoids than apolar extract (Rebaya *et al.*, 2014; Dewi *et al.*, 2020), this is in agreement with the polar nature of flavonoids (Kohoume *et al.*, 2017). The result correspond to Dewi *et al.* (2020) study, which has the highest flavonoids content on ethyl acetate extract of *Scorodocarpus borneensis* Becc bark. Additionally, the ethyl acetate extract of mixed parts of *E. arborea* was found to be richest in terms of flavonoids (150.42 ± 1.63 µg quercetin equivalents/mg extract) contents.

The content of flavonoids in leaves was higher than in flowers. These results are similar to those reported by Rebaya *et al.* (2014). In methanolic leaves extract, this amount on flavonoids is higher than that reported by previous reported data of Marquez-Garcia *et al.* (2009) in Moroccan *E. arborea* leaves for ethanolic extract with 35 mg QE/g dry weight and slightly less than that reported by Amezouar *et al.* (2013) with 54.08 mg QE/g dry weight in the alcoholic extract of same harvested part.

Regarding to the obtained phenolic, flavonoids and condensed tannins compounds values, it is difficult to establish a correct comparison between our results and those of literature data. There are no literature data for condensed tannins contents of leaves and flowers extract, but in this work they were shown to be rich in these compounds. It was also known the phenolic content was affected by solvent polarity and a particular part of the plant (Dewi *et al.*, 2020).This may be due also to the presence of different amounts of other compounds, variation of methods of extraction, which may alter the amount of phenolics (Aryal *et al.*, 2019). Therefore, this large difference in this content can be explained by other factors such as the lowering stage, the geographical and climatic conditions of plant and can lead to their difference in their bioactivity on human health (Guendouze-Boucheffa *et al.*, 2015; Medini *et al.*, 2014).

3.3. Phytochemical composition

HPLC-MS performed on phytochemical analysis of the *E. arborea* leaves, and flowers extracts revealed the presence of epicatechin, kaempferol-3-*O*-glucoside and palmitic acid as shown in Table10. In HPLC-MS analysis, we used standards for the identification of *m/z* with high resolution and the exact retention time of each analyte after chromatographic separation.

Table 10: Chemical compounds identified in *E. arborea extracts* identified by HPLC-MS.

Peak	R_t (min)	*m/z*	Ions	Molecular formula	Suggested compound	Concentrations (µg/g dry extract)	
						CrFE	CrLE
1	3.67	289	$[M-H]^-$	$C_{15}H_{14}O_6$	Epicatechin	0.0241	0.0250
2	6.57	447	$[M-H]^-$	$C_{21}H_{20}O_{11}$	Kaempferol-3-*O*-glucoside	0.1086	0.0063
3	29.97	255	$[M-H]^-$	$C_{16}H_{32}O_2$	Palmitic acid (NMR)	0.0123	0.0127

CrLE: crude extract of *E. arborea* leaves; CrFE: crude extract of *E. arborea* flowers.

Phytochemical analysis of the hydromethanolic extract obtained from flowers and leaves extracts of *E. arborea* reveals the presence of epicatechin, kaempferol-3-*O*-glucoside, and palmitic acid were detected in (Table 10). These compounds have been identified by comparing their chromatographic retention times (RT) and mass spectral data *(m/z)* with those of standards of phenolic compounds. Chromatograms obtained for the *Erica arborea* show that the peaks labeled1, 2 and 3 in Figure 16 correspond to epicatechin, kaempferol-3-*O*-glucoside, and palmitic acid, respectively.

The (-)-epicatechin (0.014 %) was found by Ay *et al.* (2007) in ethyl acetate aerial part extract of *E. arborea* (Ay *et al*., 2007). According to Márquez-García *et al.* (2009), epicatechin was also (Márquez-García *et al*., 2009) observed in the leaves of species, including *E. andeval*ensis, *E. australis*, and *E. arborea*. According to Amroun *et al.* (2021), flavonoid glycosides are the most abundant in this plant. On the other hand, Bekkai *et al.* (2022) detected kaempferol-3-*O*-glucoside in three different *E. arborea* species (Bekkai *et al.*, 2022). Identification of kaempferol 3-*O*-glucoside is also reported among thirty three flavonoid glycosides in the leaves of *E. arborea* by a previous report of Zengin *et al.* (2019), in which 72 different phenolic compounds were identified (21 phenolic acids, 27 flavonoid glycosides and 9 flavonoid aglycones, 6 flavan-3-ols, and proanthocyanidins).

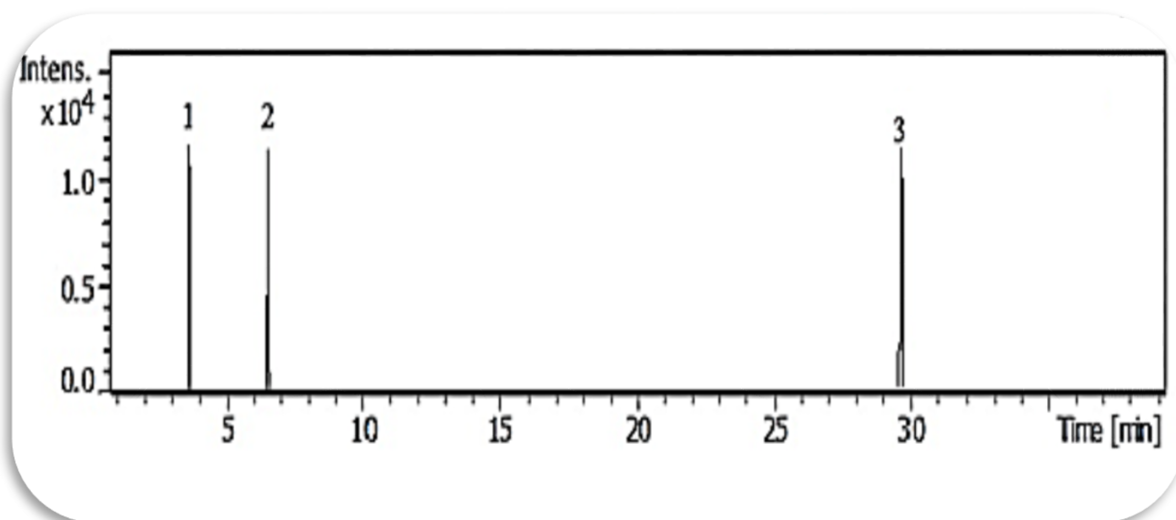

Figure 16: Chromatograms of phenolic compounds revealed in hydromethanolic extracts from leaves, and flowers of *E. arborea*.

4. Biological activities

4.1. Antioxidant activity

Due to the complex nature of phytochemicals and their interactions, the importance of using various methods based on different mechanisms for a comprehensive study of the antioxidant properties of plant extracts has been argued (Bekkai *et al*., 2022). The effect of extracts at different concentrations was studied for their ability of hydrogen or electron transferring ability measured using DPPH and reducing power tests. Among other methods, these methods are very suitable and widely used for determining the antioxidant potency of plant extracts (Chaouche *et al*., 2018; Aryal *et al*., 2019; Phuyal *et al*., 2020).As it was known, the lower the IC_{50} value the higher the antioxidant capacity of the plant extract (Pharm *et al*., 2020).

4.1.1. DPPH Radical-scavenging

The purple colored DPPH radical is capable turning to the colored-yellow as well as transformed into DPPH-H when interacted with antioxidant components presents in extracts (Trinh *et al*., 2020; Baliyen *et al*., 2022). The formation of yellow colorless, diphenylpicrylhydrazine in solution can be quantified spectrophotometrically, which DPPH radical showed a maximum absorption at 515-528 nm (Karbab *et al*., 2019).

All the examined extracts were able to reduce DPPH by donation of a hydrogen atom. The results (Figure 17) revealed that all extracts scavenged the DPPH radical with an IC_{50} values varying from 38.18 to 60.16 µg/mL for leaves and from 17.72 to 65.29 µg/mL for flowers. All these values are lower than synthetic drug BHT with an IC_{50} value of 87.65 µg/mL. The highest DPPH radical scavenging activity was exhibited with EaE of flowers with IC_{50} values of 17.72± 0.00 µg/mL, following by CrE of flowers with IC_{50} values of 24.81± 0.00 µg/mL. The lowest values was obtained by ChE of leaves and flowers.

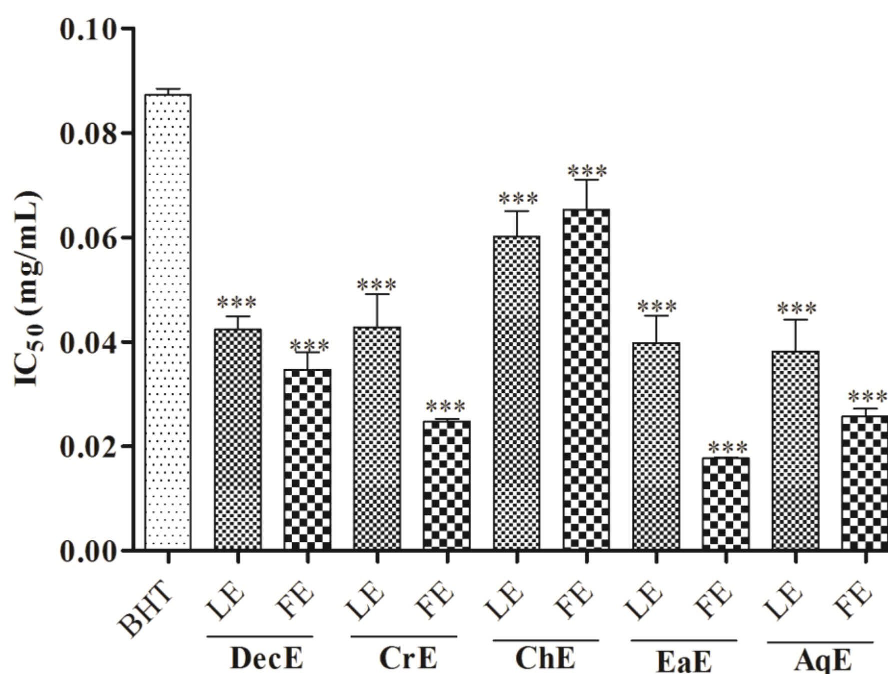

Figure 17: DPPH radical scavenging activity of leaves and flowers extracts of *E. arborea*. Data are presented as IC_{50} values. Each value represents the mean ± SD (n = 3). ***: $P \leq 0.001$.

The scavenging activity against DPPH of all extracts with different polarities of the aerial parts of *E. arborea*, native to Turkey were investigated in study of Köroğlu *et al.* (2018) in the same following order obtained in our study especially for flowers: EaE> AqE> CrE>ChE. Also, all extracts used in previous study exhibited strong antioxidant activities (Köroğlu *et al.*, 2018).

Hence, the strong antioxidant activity of *E. arborea* extracts of different vegetal parts could be due to their richness on natural antioxidant substances (Luis *et al.*, 2011; Köroğlu *et al.* 2018). Shrubs of the family of Ericaceae are known as natural sources of bioactive compounds associated to their

phenolic compounds composition (Köroğlu *et al*., 2018; Bekkai *et al*., 2022). The high phenolic content found in these species is thought to be linked to their strong free-radical scavenging effects and potential health related to therapeutic functions (Ay *et al*., 2007; Marquez- Garcia *et al*., 2009; Guendouze *et al*., 2015; Köroğlu *et al*., 2018). Some researchers reported that there is a strong correlation between total phenolic contents and the antiradical effectiveness of extracts (Köroğlu *et al*., 2018; Hmaidosh *et al*., 2020). This correlation is consistent with the current study, particularly in the EaE and ChE for polyphenols content.

The data obtained from this study reveal that EaE of flowers exhibited the most powerful antiradical effect than that of other extracts in different solvent extraction. This may be suggested by the kind of antioxidant phytochemicals such as flavonoids and phenolic compounds present in this sub-fraction as well as the presence of other natural products. Also, the same extract marked high amount of total phenols and flavonoids (Table 9). According to Sannigrahia *et al*. (2010), the low IC_{50} value of ethyl acetate fraction of *Enhydra fluctuans* Lour is due to presence of high polyphenols and flavonoids. Moreover, in the study of Ay *et al*. (2007), the ethyl acetate extract of mixed parts (leaves and flowers) of *E. arborea* showed the highest phenolic compounds and antioxidant capacity than other extracts in the DPPH assay even higher than BHT, used as reference compound.

4.1.2. Reducing power of extracts

The reduction ability of reductants or extracts under acidic conditions indicated by the transformation of Fe (III) to it Fe (II) by giving away an electron. The yellow color test solution changes to green and blue depending on the concentration which can be monitored by measurement of blue color at 700 nm (Karbab, 2020). In the current test, the reducing power ability was determined for all extracts of *E. arborea* (Figure 18), all the extracts, except chloroform extract, displayed good reducing power by the reduction of Fe^{3+}/ferricyanide to Fe^{2+}-ferrozine complex by electron donation capacity. The effect of the antioxidant activity of the samples differs according to the nature of the solvent used which could be due to different antioxidant compounds extractable (Chaouche *et al.,* 2018; Köroğlu *et al*. 2018).

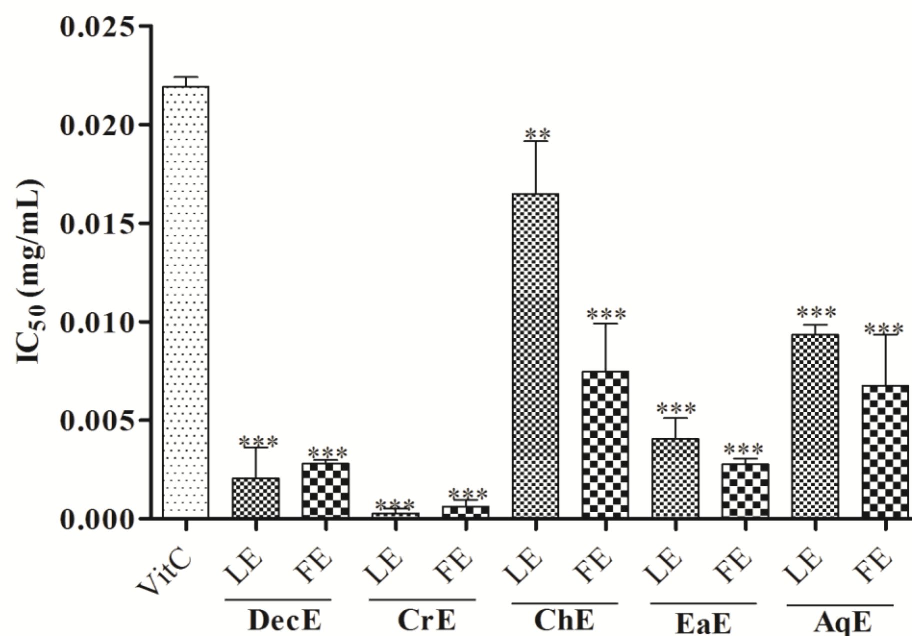

Figure 18: Reducing power of leaves and flowers extracts of E. *arborea*. Data are presented as IC_{50} values. Each value represents the mean SD (n = 3). **: $P \leq 0.01$, ***: $P \leq 0.001$.

The reducing power of the leaves is ranged from values of 0.29 to 15.12 µg/mL, whereas, the IC_{50} of flowers is ranged from values of 0.62 to 7.47 µg/mL. In comparison to ascorbic acid, the greatest reducing antioxidant power was recorded for CrE of leaves, followed by CrE of flowers with an $IC_{50} =$ 0.29 µg/mL and 0.62 µg/mL, respectively. Whereas, ChE of leaves displayed weak reducing properties. The trend of the total reducing power of the all extracts was; CrE> EaE > AqE > DecE>ChE.

In FRAP assay, the CrE presented the highest result and the ChE presented the lowest one (Aadesariya *et al.*, 2017). These results were in agreement with those found by Chaouche et al. (2018) on aerial part from *Teucrium polium* which reported that methanolic extract exhibited highest reducing power ability when compared to other extracts. In comparison to the previous study of Amezouar *et al*. (2013), which studied leaves of *E. arborea* macerated by methanol, it IC_{50} value is lower than that obtained by the present study. Also, another investigation on the dried leaves of same plant, macerated in methanol showed higher IC_{50} about 154.73 µg/mL (Suna *et al.*, 2018). In other study, the ethanolic extract of *E. arborea* exhibited notable antioxidant activity in FRAP assay with the high total polyphenols and tannins values (Pavlovic *et al.*, 2014).

In recent years the powerful antioxidant capacity of the flavonoids has been attracting much attention (Köroğlu et al., 2018). Ethyl acetate fraction of flowers possessed the considerable flavonoid substances and antioxidant activity. There are several studies that reported a significant correlation between flavonoid and antioxidant activity by DPPH method (Aryal *et al*., 2019; Dewi *et al*., 2020). The ethyl acetate extract of aerial parts of *E. arborea* was found to be richest in terms of phenolic especially flavonoids contents which exhibited the highest DPPH antioxidant activity (Ay *et al*., 2007; Köroğlu *et al*., 2018). Furthermore, in plates sprayed with 1% vanillin-H_2SO_4 analysis detected mainly flavonoids such as kaempferol and luteolin in ethyl acetate extracts of aerial part of *Erica* species (Köroğlu *et al*., 2018).

In previous study of Nazemiyeh *et al*. (2008) the methanol extract of leaves of *E. arborea* afforded five flavonoids and exhibited a higher antioxidant activity than the propyl gallate used as standard. Pavlović *et al*. (2014) explained the antioxidant capacity of *Erica* species, macerated in ethanol by the presence of kaempferol-3-O-β-D-galactoside and quercetin. Flavonoids in the middle of natural phenolic compounds were known to possess the most potent radical-scavenging and to act in different mechanisms in the regulation of oxidative stress (Dewi *et al*., 2020; Baliyen *et al*., 2022). Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Adaramola *et al*., 2017).

According to multiple reports in the literature, the structural characteristics of polyphenols is certainly the most important parameter, for free radical scavenging and reducing power capacities (Guendouze-Boucheffa *et al*., 2015; Chaouche *et al*., 2018; Aryal *et al*., 2019; Dewi *et al*., 2020; Phuyal *et al*., 2020)**.** Therefore**,** phenolics possess one or more aromatic rings, and one or more hydroxyl groups that are disposed to donate a hydrogen atom or an electron to a free radical (Chaouche *et al*., 2018; Aryal *et al*., 2019). The antioxidant activity of flavonoids isolated by Nazemiyeh *et al*. (2008) is a consequence of the presence of the phenolic moieties in the structures (Nazemiyeh *et al*., 2008). It was demonstrated the particular substitution pattern of free groups on the flavonoid skeleton influenced the potential of antioxidant activity. Thus, the presence of hydroxyl and-carbonyl group in the favonoid skeleton resulted in high FRAP potential and the presence of 2,3-double bond in conjugation with the 4-oxo function in the C-ring resulted potent radical scavenging ability (Afsar *et al*., 2018).

4.2. Antibacterial Activity

4.2.1. Growth inhibition of bacteria

Antibiotic resistance is a problem that continues to challenge the health care sector in a large part of the world in both developing and developed countries. The emergence and spread of multidrug resistant pathogens have substantially threatened the current antibacterial therapy (Manandhar *et al.*, 2019). This has necessitated a search for a new source of antimicrobial substance such as medicinal plants as they produce a variety of bioactive compounds known therapeutic properties (Manandhar *et al.*, 2019; Kebede *et al.*, 2021). The antibacterial activity of all extracts was determined initially by the disc diffusion method against humans pathogens including six reference bacteria. These bacterial strains are Gram-positive and Gram-negative species frequently encountered infectious diseases.

Agar disk diffusion testing is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Generally, the antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganisms (Balouiri *et al.*, 2016). The sensibility of these bacteria against tested extracts was measured and diameters of inhibition zones were energised. According to the classification of Bensemir *et al.* (2006), the bacterial strain is considered as not sensitive for diameter of zone of inhibition less or equal as 8 mm, sensitive for a diameter between 8-15 mm and very sensitive for diameter more 15mm.The results of the diameters of inhibition zones are shown in the Table11 and 12 , illustrated in Figure 19, 20, 21 and 22.

There are a numbers of factors that could influence the results of the disc diffusion assay. Firstly, the diameter of the zone is affected by the rate of diffusion of antimicrobial compound. Another important factor is the standardisation of the inoculum size to 0.5 Mc Farland turbidity (Razmavar *et al.*, 2014). Antibacterial assay showed a positive correlation of the inhibitory zone of extracts against the bacteria with the applied concentrations (Rita *et al.*, 2021).

Table 11: Antibacterial activity of leaves extracts of *E. arborea* per mm against tested microorganisms.

Extracts	Concentration (mg/mL)	Bacteria strains/diameter of zone (mm)					
		B. cereus	***E. coli***	***M. luteus***	***P. aeruginosa***	***S. gallinarum***	***S. aureus***
CrE	100	$11.73 \pm 0.33^{***}$	$10.97 \pm 2.78^{***}$	$10.63 \pm 0.13^{***}$	$10.11 \pm 2.14^{***}$	$10.38 \pm 0.05^{***}$	$11.94 \pm 10.25^{***}$
	50	$9.69 \pm 0.14^{***}$	$10.22 \pm 0.97^{***}$	$9.56 \pm 0.11^{***}$	$10.02 \pm 1.01^{***}$	$9.47 \pm 0.08^{***}$	$10.25 \pm 0.06^{***}$
	25	$9.19 \pm 0.23^{***}$	$9.27 \pm 0.65^{***}$	$9.06 \pm 0.26^{***}$	$9.55 \pm 0.33^{***}$	$8.26 \pm 0.15^{***}$	$9.67 \pm 0.38^{***}$
	12.5	$7.87 \pm 0.11^{***}$	$8.6 \pm 0.92^{***}$	$8.1 \pm 0.09^{***}$	$9.12 \pm 1.59^{***}$	$7.67 \pm 0.04^{***}$	$9.18 \pm 0.25^{***}$
DecE	100	$10.37 \pm 0.50^{***}$	$10.46 \pm 0.65^{***}$	$10.87 \pm 0.46^{***}$	$10.72 \pm 0.86^{***}$	$10.89 \pm 1.43^{***}$	$11.40 \pm 0.32^{***}$
	50	$9.08 \pm 0.12^{***}$	$9.23 \pm 0.33^{***}$	$8.42 \pm 0.36^{***}$	$9.54 \pm 0.31^{***}$	$8.82 \pm 0.43^{***}$	$9.85 \pm 0.26^{***}$
	25	$8.18 \pm 0.30^{***}$	$9.07 \pm 0.10^{***}$	$8.38 \pm 0.77^{**}$	$9.07 \pm 0.11^{**}$	$8.66 \pm 0.36^{**}$	$9.65 \pm 0.27^{**}$
	12.5	$7.85 \pm 0.09^{***}$	$8.09 \pm 0.11^{***}$	$7.12 \pm 0.35^{***}$	$8.01 \pm 0.14^{***}$	$7.37 \pm 0.44^{***}$	$8.7 \pm 0.25^{***}$
ChE	100	/	/	/	/	/	$10.31 \pm 1.29^{***}$
	50	/	/	/	/	/	$9.37 \pm 0.42^{***}$
	25	/	/	/	/	/	$9.51 \pm 0.30^{**}$
	12.5	/	/	/	/	/	$8.50 \pm 0.71^{**}$
EaE	100	$13.15 \pm 0.29^{***}$	$13.51 \pm 1.18^{***}$	$7.52 \pm 0.67^{***}$	$13.11 \pm 0.64^{***}$	$12.14 \pm 0.11^{***}$	$12.40 \pm 0.34^{***}$
	50	$10.93 \pm 0.08^{***}$	$10.82 \pm 0.55^{***}$	$7.18 \pm 0.86^{***}$	$11.88 \pm 0.88^{***}$	$10.55 \pm 0.09^{***}$	$10.98 \pm 0.45^{***}$
	25	$9.34 \pm 0.18^{***}$	$9.84 \pm 0.37^{***}$	$7.00 \pm 0.00^{**}$	$9.37 \pm 0.09^{**}$	$9.14 \pm 0.07^{**}$	$9.19 \pm 0.17^{**}$
	12.5	$8.04 \pm 0.04^{***}$	$9.60 \pm 0.27^{***}$	/	$9.20 \pm 0.83^{***}$	$7.81 \pm 0.09^{***}$	$8.19 \pm 0.27^{***}$
AqE	100	/	/	$12.32 \pm 1.83^{***}$	$10.89 \pm 0.78^{***}$	/	/
	50	/	/	/	$9.46 \pm 0.13^{***}$	/	/
	25	/	/	/	$9.33 \pm 0.18^{***}$	/	/
	12.5	/	/	/	/	/	/
Gentamicin	0.120	27.96 ± 1.71	27.34 ± 0.29	26.48 ± 2.00	27.28 ± 0.50	26.35 ± 1.21	27.28 ± 0.27

/: no inhibition zone ; DecE: decoction extract; CrE: crude extract; ChE: chloroform extract; EaE: ethyl acetate extract, AqE: aqueous extract. Each value represents the mean ± SD (n = 3). ***. $P \leq 0.001$.

Table 12: Antibacterial activity of flowers extracts of *E. arborea* per mm against tested microorganisms.

Extracts	Concentration (mg/mL)	Bacteria strains/diameter of zone (mm)					
		B. cereus	*E. coli*	*M. luteus*	*P. aeruginosa*	*S. gallinarum*	*S. aureus*
CrE	100	12.91 ± 0.04***	13.17 ± 0.14***	7.73 ± 0.92***	13.88 ± 0.28***	12.92 ± 0.09***	12.08 ± 0.29***
	50	10.78 ± 0.07***	11.08 ± 0.77***	7.27 ± 0.17***	10.59 ± 0.29***	11.41 ± 0.56***	10.68 ± 0.26***
	25	9.53 ± 0.12***	9.85 ± 0.09***	7.14 ± 0.77***	10.00 ± 0.07***	10.18 ± 0.48***	9.66 ± 0.01***
	12.5	8.59±0.09***	9.64 ± 0.14***	6.95 ± 0.26***	9.93 ± 0.02***	7.31 ± 0.11***	8.90 ± 0.02***
DecE	100	11.66 ± 0.33***	11.27 ± 0.08***	7.27 ± 0.18***	10.99 ± 0.35***	12.72 ± 0.06***	11.04 ± 0.14***
	50	10.61 ± 0.50***	9.89± 0.07***	7.14 ± 0.77***	9.39 ± 0.47***	11.45 ± 0.51***	9.54 ± 0.81***
	25	10.32 ± 0.72***	8.23 ± 0.04***	/	8.14 ± 0.22***	10.93± 0.03***	8.18 ± 0.00***
	12.5	/	/	/	/	9.77± 0.23***	/
ChE			/	/	/	/	/
EaE	100	9.68 ± 1.47***	/	16.30 ± 0.04***	17.04 ± 1.48***	/	/
	50	9.47 ± 2.08***	/	15.54 ± 0.89***	15.08 ± 0.99***	/	/
	25		/	14.23 ± 0.13***	13.85 ± 0.14***	/	/
	12.5		/	10.88 ± 0.27***	11.15 ± 0.07***	/	/
AqE	100	/	/	12.25 ± 0.20***	12.25 ± 0.15***	/	/
	50	/	/	10.24 ± 0.34***	11.24 ± 0.64***	/	/
	25	/	/	9.98 ± 0.47***	9.83 ± 0.25***	/	/
	12.5	/	/	9.00 ± 0.00***	9.01± 0.02***	/	/
Gentamicin	0.120	27.96 ± 1.71	27.34 ± 0.29	26.48 ± 2.00	27.28 ± 0.50	26.35 ± 1.21	27.28 ± 0.27

/: no inhibition zone ; DecE: decoction extract; CrE: crude extract; ChE: chloroform extract; EaE: ethyl acetate extract, AqE: aqueous extract. Each value represents the mean ± SD (n = 3). ***. P ≤ 0.001.

The results of previous studies (Balouiri *et al*., 2016; Deyno *et al*., 2021) were in agreement with those of the present study where extraction by different solvents showed different activities. It can be noted that the crude extracts (CrE) of all plant parts, exhibited varying degrees of antibacterial activity against both Gram-negative and Gram-positive bacterial strains tested, with zones of inhibition in the range of 10.11-11.94 mm for leaf extract (LE), 7.73 -13.88 mm for flower extract (FE), which are presented in Figure 19. The CrE of flowers presented a strong activity against *P. aeruginosa*, *E. coli* and *S. gallinarum* with diameter of inhibition zone of 13.88, 13.17 and 12.92 mm, respectively. However, *M. luteus* appears to be the most resistant strain to the same extract with a zone of inhibition of 7.73 mm.

Our results are disaccord with those found by previous studies of Pavlovic *et al*. (2014), Guendouze-Boucheffa *et al*. (2015) and Yaici *et al*. (2019), those have reported that the growth of Gram-positive bacteria is more sensible about the inhibitory effect of *E. arborea* extracts. On the other hand, our results corroborated with the results of previous Turkish study established by Kivçak *et al*. (2013) about the antibacterial activity of the methanolic extract of aerial parts of *E. arborea* from Turkish provenance against the growth of Gram-negative *E. coli* ATCC29998 and *E. coli* ATCC 11230 G and not against *P. aeruginosa* ATCC2753. Yaici *et al*. (2019) indicated that the antibacterial efficiency of *E. arborea* extracts against *S. aureus* and *Bacillus subtillus* due to their presence of hydroquinone as antibacterial factor in treatment of urinary tract disorders.

The antibacterial effectiveness of the methanolic extract of studied aerial parts of *E. arborea* against bacteria could be attributed to the action of diverse varieties of bioactive compounds (Ćetković *et al*., 2007). Moreover, the variability in bacterial behavior of tested bacteria to extracts can supposed to the quality of phenolic or no phenolic constituents (Banothu et al., 2017), confirmed by previous study of Bitchagno *et al.* (2015), which was characterized the new antibacterial biflavonoid purified compound from the whole plant of *Erica mannii,* Ericaceae family. The high molecular weight of this polar substance influenced moderate antibacterial activity against *E. coli* bacteria, due to it permeability not easy to enter cell exterior. Although, the second purified compound, ericoside had a significantly greater activity against the same bacteria (Bitchagno *et al*., 2015). Additionally, the synergistic effect between different phenolic compounds or non-phenolic compounds may induce antibacterial potential (Ćetković *et al*., 2007; Sadeq *et al*., 2020).

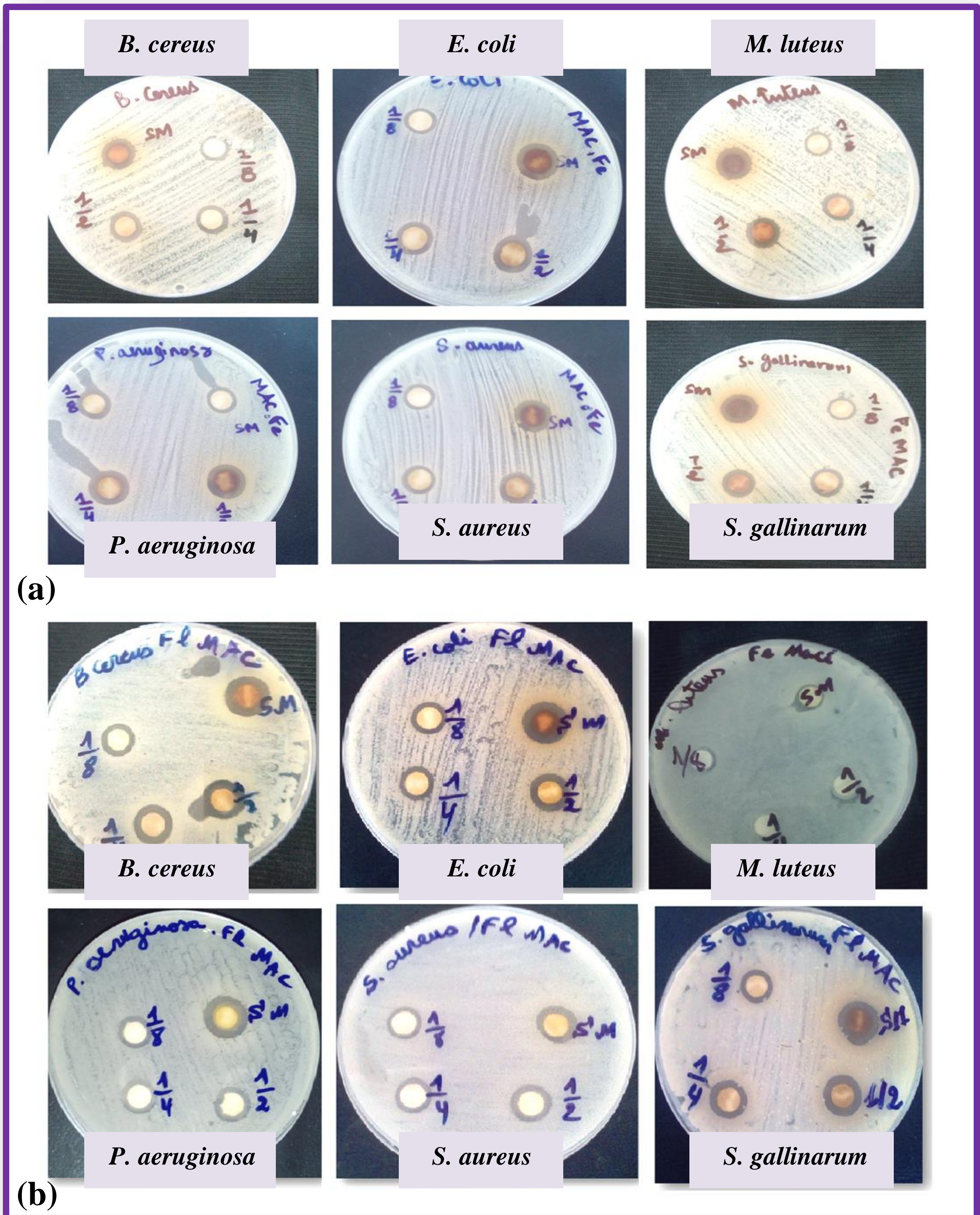

Figure 19: Antibacterial activity of crude extract (CrE) of leaves **(a)** and flowers **(b)** against *B. cereus*, *E. coli*, *M. luteus*, *P.aeruginosa*, *S. aureus* and *S. gallinarum*.

Antibacterial potential of *E. arborea* methanolic extracts could be correlated with the presence of bioactive phytochemicals identified in different vegetal parts such as epicatechin, kaempferol-3-*O*-glucoside. Indeed, the phytochemical analysis of green tea and many plants extract showed the presence of epicatechin, which appear greater antibacterial action against Gram-positive bacteria such as *Staphylococcus aureus* rather than Gram-negative ones such as *Escherichia coli* (Ajiboye *et al.*, 2016; Gomes *et al.*, 2018). Thus, the association of catechins with proteins of cells emphasizes the antibacterial function (Gomes *et al.*, 2018). Catechin and epicatechin can also neutralized endotoxin of Gram negative bacteria such as lipopolysaccharide (Delehanty *et al.*, 2007; Raygaert *et al.*, 2014).

According to the authors, kaempferol-3-*O*-glucoside isolated from *Annona muricata* and *Helichrysum compactum* extracts could be responsible for their antibacterial effects (Jasmine Mary and Merina, 2014; Taiwo, 2019). Many of the bacterial strains commonly encountered by humans are killed by flavonoids (Guendouze-Boucheffa *et al.*, 2015). It is known that the inhibition mechanism of flavonoids in medicinal plant was influenced by number and position of their hydroxyl groups (Bitchagno *et al.*, 2015); which can gives defense in microbial infections by increase in the permeability of the internal bacterial membrane, disorder of membrane potential and enzymes inhibition (Cushnie and Lamb, 2005; Cushnie and Lamb, 2011). According to Rita *et al.* (2016) the activity of flavonoids and phenols as antibacterial is caused by the formation of complexes with bacterial proteins through hydrogen bonds, covalent bonds and hydrophobic bonds, so as to deactivate enzymes from bacteria (Rita *et al.*, 2021). It is important to mention that the permeability of the bacterial cell to the flavonol glycosides is one of the factors that determine their antibacterial effects (Yahia *et al.*, 2020).

Additionally, the antibacterial properties are probably due to fatty acids, antimicrobials agents non phenolic and well known to treat numerous bacterial infections (Yoon *et al.*, 2018; Casillas-vargas *et al.*, 2021). Many studies have demonstrated that palmitic acid and oleic acid is the main component of a variety of extracts. (Babaiwa *et al.*, 2017; Xie *et al.*, 2021). Thus, fatty acids have been associated with antibacterial activity due to the ability of fatty acids to intercalate into the bacterial cell membrane causing increased fluidity, permeability changes and consequently the lyse of the unstable bacterial cells (Babaiwa *et al.*, 2017).

The results indicated that ethyl acetate extracts of *E. arborea* showed varying degree of inhibitory and showed much greater activity towards various microbes tested. The ethyl acetate extract (EaE) of leaves of *E. arborea* showed remarkable antibacterial activities against all tested bacterial strains, with zones of inhibition in the range of 7.52 to 13.51 mm, which presented Figure 20. The EaE of leaves presented a strong activity against *E. coli* and *B. cereus* with diameter of inhibition zone of 13.51 and 13.15 mm, respectively. However, *M. luteus* appears to be the most resistant strain to the same extract with a zone of inhibition of 7.52 mm.

The EaE of flowers extract presented only antibacterial activity against *P. aeruginosa*, *M. luteus* and *B.* cereus. The ethyl acetate extract of flowers showed 17.04 mm zone of inhibition against gram negative *P. Aeruginosa* and 16.30 mm zone of inhibition against *M. luteus* gram positive in which good antibacterial activity were indicated, which presented in Figure 20.

The highest antibacterial activity of EaE compared to CrE and other fractions were also reported in previous studies (Deyno *et al*., 2021; Nie *et al*., 2021; Rita *et al*., 2021). Some bioactive components of these plants may differ in their solubility depending on the extractive solvents used (Lee *et al*., 2014). Thus, the fractionation may be enhance concentrations and activities of antibacterial principles in these fractions (Tamokou *et al*., 2012). As a result, the ethyl acetate fraction of *Abrus cantoniensis* and milk banana peels were the most actives in inhibiting the growth of both bacteria *S. aureus* compared to the other extracts (Nie *et al*., 2021; Rita *et al*., 2021).

The promising antibacterial effect of ethyl acetate fraction could be attributed to the action of its chemical components (Ghaima *et al*., 2013; Pu *et al*., 2014; Babaiwa *et al*., 2017; Deyno *et al*., 2021; Guo *et al*., 2022). Additionally, polar flavonoids were proved to be the predominant components in ethyl acetate extract of several medicinal plants (Chen *et al*., 2012; Ghasemian-Yadegari *et al*., 2019; Guo *et al*., 2022; Rita *et al*., 2021). Furthermore, ethyl acetate extracts proved to be rich source of fatty acids by previous authors which speculated to be responsible for the observed antibacterial activity (Babaiwa *et al*. (2017). Antibacterial activity of ethyl acetate extract of *Citrullus lanatus* was reported in connection with the presence of fatty acids such as oleic acid as the predominant one (Babaiwa *et al*., 2017). Because their lipophilic nature, fatty acids will most likely be found in the less polar ethyl acetate extracts than polar methanol extracts (Borquaye *et al*., 2016) and probably account for the high activity of ethyl acetate extract observed in this work.

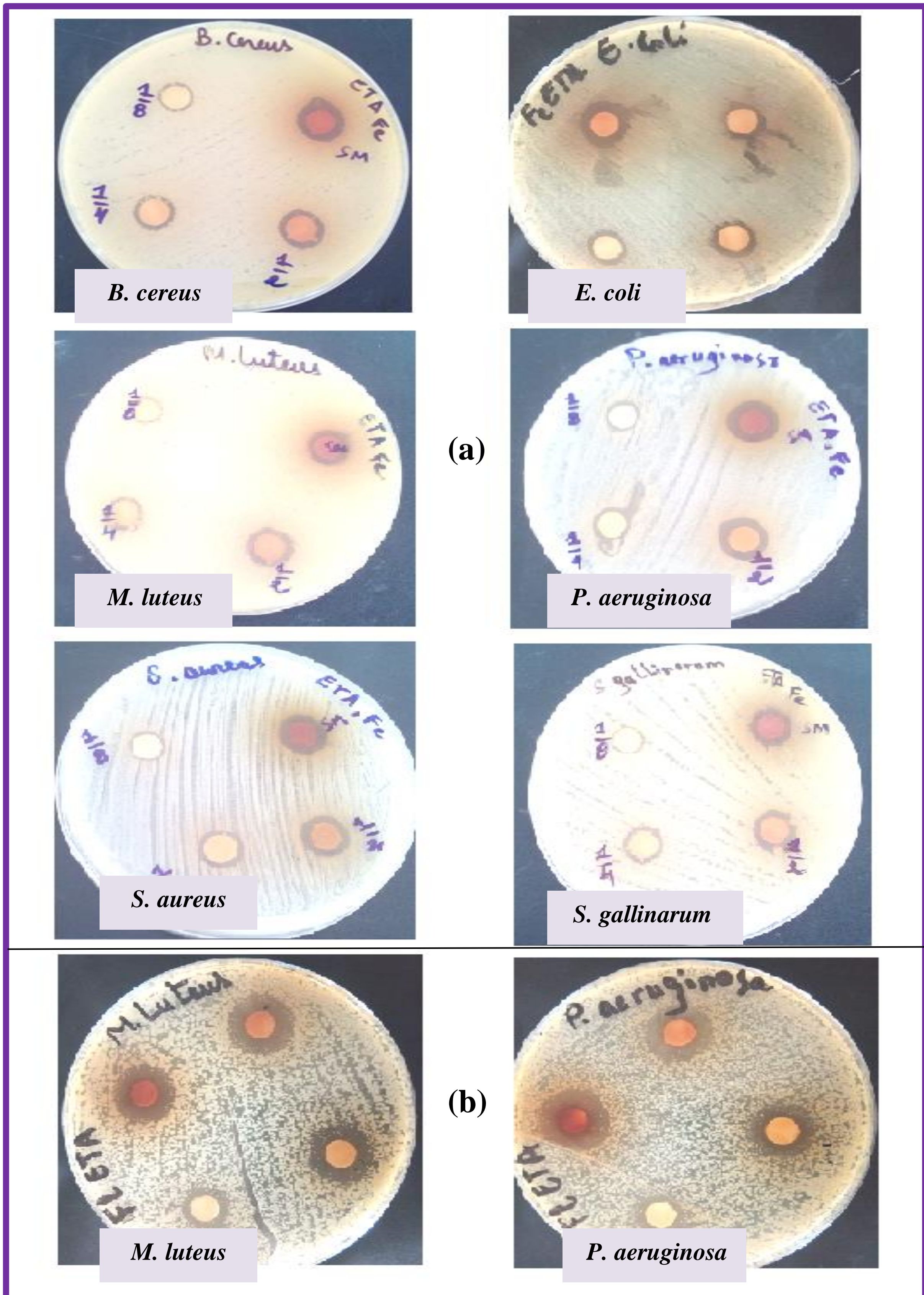

Figure 20: Antibacterial activity of ethyl acetate (EaE) of leaves **(a)** and flower **(b)** against bacteria.

However, the aqueous extracts (AqE) from leaves and flowers of *E. arborea* only showed antibacterial activities against *M. luteus* and *P. aeruginosa* (Figure 21). Only chloroform extract (ChE) of leaves showed antibacterial activity against *S. aureus.* Contrary to results of Pavlovic *et al.* (2014) where reported that aqueous ethanolic extract of *E. arborea* leaves was no active against *S. aureus*. The difference in results could be due to the use of plant extract in high concentration compared to that used by them. This difference may be also due to the use of different solvents system. It has widely observed and accepted that the medicinal value of plant that dissolve in different solvent system (Manandhar et al., 2019). Our results are in agreements with those found by previous studies of Guendouze-Boucheffa et al. (2015) and Yaici et al. (2019) about inhibitory effect against gram-positive *S. aureus.*

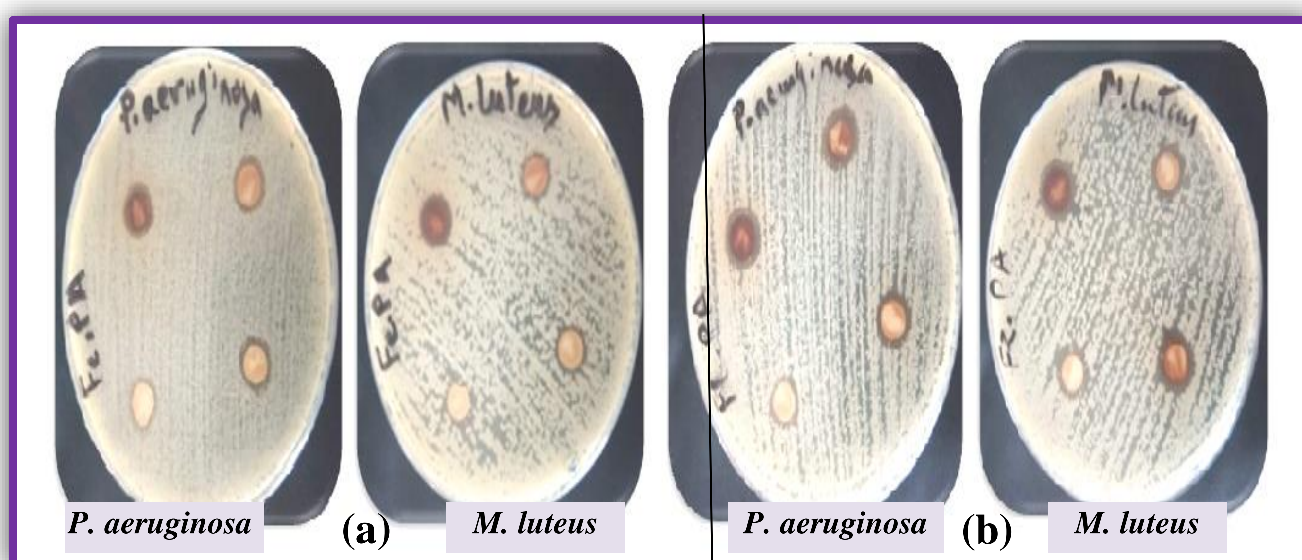

Figure 21: Antibacterial activity of aqueous extract (AqE) of leaves **(a)** and flowers**(b)** against *P. aeruginosa* and *M. luteus.*

We observed that the Dec E of *E. arborea* L showed remarkable antibacterial activities against all tested bacterial strains, with zones of inhibition in the range of 10.37 to 11.40 mm for LE, 7.27 to 12.72 mm for FE, which are presented Figure 22. Also, we found that *M. luteus* showed resistant to the DecE of flowers with a zone of inhibition of 7.27 mm. However, it is the ease of use that makes water the most used solvent for bioactive compounds in traditional remedy preparations (Shale *et al.*, 1999). Antibacterial activity observed by decoction extracts may be due to presence of compounds, which are polar in nature (Bibi *et al.*, 2011). Also, chlorogenic acid and six flavonoids were identified through HPLC-DAD-MS phytochemical analysis from decoction extracts of *E. arborea*. Also, Quercetin-3-O-glycoside and myricetin O-glycoside were the major components in same

extract (Amroun *et al*., 2021).Antibacterial potency of diverse DecE of *E. arborea* against all bacteria strains mayjustifytheir remedial act in the treatment of diverse urinary infections mentioned in traditional medicine (Kivçak *et al*., 2013).

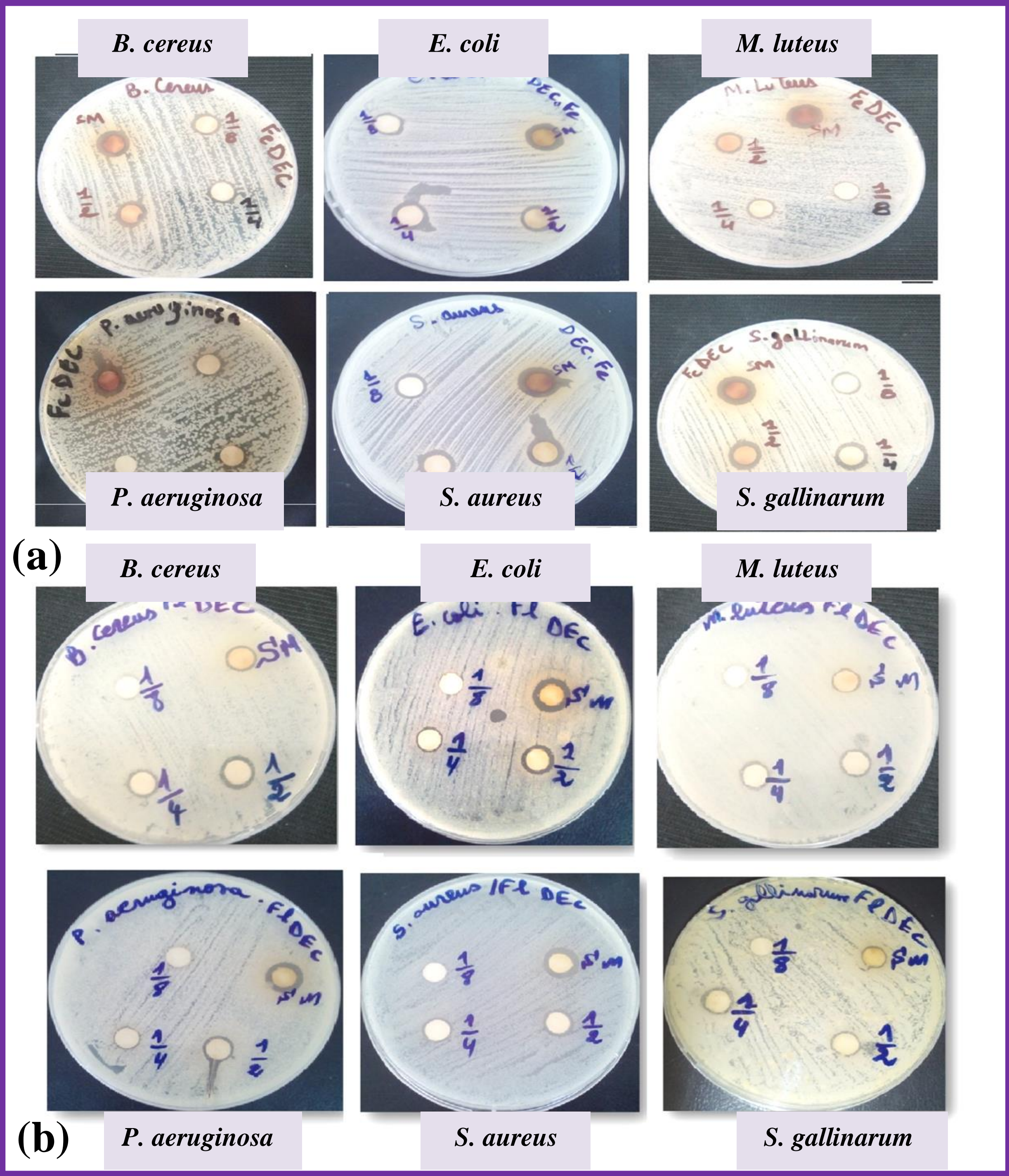

Figure 22: Antibacterial activity of decoction extract (DecE) of leaves **(a)** and flowers **(b)** against *B. cereus*, *E. coli*, *M. luteus*, *P. aeruginosa*, *S. aureus* and *S. gallinarum*

4.2.2. Minimum inhibitory concentration

Our preliminary antibacterial studies with disk diffusion method suggested on interesting profile of antibacterial effect against bacteria. The minimum inhibitory concentration (MIC) and mnimum bactericidal concentration (MBC) assays were used to evaluate the efficiencies of antimicrobial agents in the extracts of *E. arborea* and also to characterize the nature of the effect revealed by the extracts on each strain (Harchaoui *et al*., 2022). The antimicrobial activity of a plant extract is considered to be highly active if the MIC <0.1 mg/mL; active when 0.1 ≤ MIC ≤0.512mg/mL; moderately active when 0.512< MIC ≤2.048 mg/mL; weakly active if MIC > 2.048 mg/mL and not active when MIC > 10 mg/mL (Tagousop *et al*., 2018). Also, The bactericidal or bacteriostatic activity of extracts against bacteria was evaluated using MBC/MIC ratio. The MIC, MBC and MBC/MIC ratio of the extracts against all tested pathogenic bacteria are summarized in Table 13 and Table 14.

The high MIC values obtained from CrE from L (leaves) and F (flowers) were 1.60 ± 0.25, 2.14 ± 060 and 2.45 ± 0.49 mg/mL against *M. luteus* and *P. aeruginosa*, especially by the FE with MIC value of 1.60 ± 0.25 mg/mL and MBC value of 2.50 ± 0.29 mg/mL, which presented an interesting bactericidal action. In addition, the LE showed excellent MIC values toward *M. luteus* and *P. aeruginosa*, with 2.14 ± 0.60 and 2.45 ± 0.49 mg/mL, respectively. As well, the MBC values of crude extract of leaves were of 3.12 ± 0.72 mg/mL, and 3.42 ± 0.56 mg/mL against the same bacteria, respectively.

The CrE of flowers exhibited an encouraging value of 1.6 mg/mL against *M. luteus* compared to that recorded by gentamicin value at 1.01 mg/mL. In this respect, Guendouze-Boucheffa and colleagues (2015) showed that the methanol flowering extract of *E. arborea* has a significant effect against only Gram-positive *S. aureus* bacteria with an MIC value of 500 µg/mL, contrary to Gram-negative *E. coli* and *P. aeruginosa* bacteria. The variability in the results with Guendouze-Bouchefa *et al*. (2015) may be due to the different parameters such as the harvest localization, the part used and the extraction technique. Thus, the author in the respective study used dilapidation by hexane, the additional step before extracting of compounds presents in plant, which needs to removing probably undesirable interferences such as fats, terpenes, and pigments (Alara *et al*., 2021).

Table 13: MIC, MBC and MBC/MIC ratio of leaves extracts against the tested bacteria

Extracts		Bacteria (mg/mL)					
		B. cereus	*E. coli*	*M. luteus*	*P. aeruginosa*	*S. aureus*	*S. gallinarum*
DecE	MIC	$12.13 \pm 2.19^{***}$	$34.30 \pm 7.78^{***}$	1.07 ± 1.14^{ns}	$12.40 \pm 3.85^{***}$	$3.02 \pm 0.79^{*}$	$8.87 \pm 1.07^{**}$
	MBC	$13.89 \pm 2.46^{***}$	$38.34 \pm 9.38^{***}$	1.11 ± 1.40^{ns}	$15.54 \pm 4.92^{***}$	$3.50 \pm 0.87^{**}$	$10.15 \pm 0.66^{**}$
	MBC/MIC	≤4	≤4	≤4	≤4	≤4	≤4
CrE	MIC	$3.50 \pm 0.95^{**}$	6.12 ± 0.96^{ns}	2.14 ± 0.60^{ns}	2.45 ± 0.49^{ns}	$8.03 \pm 1.19^{***}$	$5.29 \pm 0.37^{*}$
	MBC	$4.77 \pm 1.11^{**}$	6.99 ± 1.00^{ns}	3.12 ± 0.72^{ns}	3.42 ± 0.56^{ns}	$9.40 \pm 1.44^{***}$	6.15 ± 0.42^{ns}
	MBC/MIC	≤4	≤4	≤4	≤4	≤4	≤4
ChE	MIC	ND	ND	ND	ND	$7.60 \pm 0.92^{***}$	ND
	MBC	ND	ND	ND	ND	$8.66 \pm 0.94^{***}$	ND
	MBC/MIC	ND	ND	ND	ND	≤4	ND
EaE	MIC	$3.15 \pm 0.28^{**}$	7.68 ± 0.42^{ns}	0.63 ± 0.48^{ns}	$10.90 \pm 4.90^{***}$	0.06 ± 0.07^{ns}	$36.30 \pm 2.77^{***}$
	MBC	$3.78 \pm 0.27^{*}$	8.69 ± 0.46^{ns}	$6.88 \pm 2.31^{***}$	$15.13 \pm 5.62^{***}$	1.43 ± 0.05^{ns}	$42.21 \pm 3.63^{***}$
	MBC/MIC	≤ 4	≤4	≥4	<4	≥4	≤4
AqE	MIC	ND	ND	1.97 ± 0.30^{ns}	2.04 ± 0.24^{ns}	ND	ND
	MBC	ND	ND	2.74 ± 0.31^{ns}	2.76 ± 0.24^{ns}	ND	ND
	MBC/MIC	ND	ND	≤4	≤4	ND	ND
Gentamicin	MIC	0.3 ± 0.00	0.5 ± 0.00	1.01 ± 0.17	$0.09 \pm 0.01^{***}$	$0.01 \pm 0.00^{***}$	0.04 ± 0.00
	MBC	0.13 ± 0.03	0.01 ± 0.00	0.39 ± 0.02	$0.08 \pm 0.06^{***}$	$0.01 \pm 0.01^{***}$	0.01 ± 0.00
	MBC/MIC	≤4	≤4	≤4	≤4	≤4	≤4

ND: no determined; AqE: Aqueous extract, CrE: crude extract, ChE: chloroform extract, DecE: decoction extract, EaE: ethyl acetate extract, MBC: Minimum bactericidal concentration; MIC: Minimum inhibitory concentration; MBC/MIC: MBC/MIC ratio. ns: no significant differences *: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$.

Table 14: MIC, MBC and MBC/MIC ratio of flowers extracts against the tested bacteria

Extracts		Bacteria (mg/mL)					
		B. cereus	*E. coli*	*M. luteus*	*P. aeruginosa*	*S. aureus*	*S. gallinarum*
DecE	MIC	5.22 ±0.92***	29.11 ± 0.25***	1.31 ± 0.25^{ns}	4.91 ± 0.91^{ns}	6.91 ± 0.91***	7.11 ± 1.92**
	MBC	6.21 ±1.22***	31.13 ± 1.27***	1.76 ± 0.26^{ns}	5.75 ± 0.60^{ns}	5.75 ± 0.60***	8.14 ± 2.03*
	MBC/MIC	≤4	≤4	≤4	≤4	≤4	≤4
CrE	MIC	6.38 ±0.86***	8.77 ± 0.94*	1.60 ± 0.25^{ns}	9.13 ± 0.34**	5.66 ± 1.09***	5.41 ± 0.27^{ns}
	MBC	6.64 ±1.58***	10.05 ± 1.07*	2.50 ± 0.29^{ns}	10.66 ± 0.34**	6.81 ± 1.31***	6.25 ± 0.25^{ns}
	MBC/MIC	≤4	≤4	≤4	≤4	≤4	≤4
ChE	MIC	ND	ND	ND	ND	ND	ND
	MBC	ND	ND	ND	ND	ND	ND
	MBC/MIC	ND	ND	ND	ND	ND	ND
EaE	MIC	1.91± 0.13^{ns}	ND	1.00± 1.40^{ns}	0.17± 0.09^{ns}	ND	ND
	MBC	2.69 ±0.20^{ns}	ND	1.93± 1.79^{ns}	0.80± 0.15^{ns}	ND	ND
	MBC/MIC	≤4	ND	≤4	>4	ND	ND
AqE	MIC	ND	ND	1.99± 0.78^{ns}	1.73± 0.23^{ns}	ND	ND
	MBC	ND	ND	2.97± 0.84^{ns}	2.22± 0.25^{ns}	ND	ND
	MBC/MIC	ND	ND	>4	>4	ND	ND
Gentamicin	MIC	0.3 ± 0.00	0.5 ± 0.00	1.01 ± 0.17	0.09 ± 0.01	0.01 ± 0.00	0.04 ± 0.00
	MBC	0.13 ± 0.03	0.01 ± 0.00	0.39 ± 0.02	0.08 ± 0.06	0.01 ± 0.01	0.01± 0.00
	MBC/MIC	≤4	≤4	≤4	≤4	≤4	≤4

ND: no determined; AqE: Aqueous extract, CrE: crude extract, ChE: chloroform extract, DecE: decoction extract, EaE: ethyl acetate extract, MBC: Minimum bactericidal concentration; MIC: Minimum inhibitory concentration; MBC/MIC: MBC/MIC ratio. ns: no significant differences *: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$.

In the decoction extraction, the plant extracts showed antibacterial activity with MIC values in the ranges of 1.07-34.30 mg/mL for (L) and 1.31-29.11 mg/mL for (F). DecE of (L) showed excellent activity toward *M. luteus*, and *S. aureus* with MIC values of 1.07 ± 1.14 and 3.02 ± 0.79 mg/mL, respectively. Furthermore, DecE of (F) showed excellent activity toward *M. luteus*, with MIC value of 1.31 ± 0.25 mg/mL and MBC value of 1.76 ± 0.26 mg/mL.

In the ethyl acetate phase, the plant extracts showed antibacterial activity with MIC values in the ranges of 0.06-36.30 mg/mL for (L) and 0.17-1.91 mg/mL for (F). EaE of (L) showed excellent activity toward *S. aureus* and *M. luteus* , MIC values of 0.06 ± 0.07 and 0.63 ± 0.48 mg/mL against the two strains; MBC 1.43 ± 0.05 and 6.88 ± 2.31 mg/mL, respectively. Furthermore, EaE of (F) showed excellent activity toward *P. aeruginosa*, *M. luteus* and *B. cereus* with MIC values of 0.17 ± 0.09, 1.00 ± 1.40 and 1.91 ± 0.13 mg/mL against the three strains; MBC 0.80 ± 0.15, 1.93 ± 1.79 and 2.69 ± 0.20 mg/mL, respectively.

Minimal inhibitory concentration values of the antibacterial activity of the aqueous extract (AqE) of the (L) showed excellent activity toward *M. luteus* and *P. aeruginosa* with MIC values of 1.97 ± 0.30 and 2.04 ± 0.24, respectively. Furthermore, AqE of (F) showed excellent activity toward *P. aeruginosa* and *M. luteus* with MIC values of 1.73± 0.23 and 1.99±0.78 mg/mL, respectively. The antibacterial potential was considered bactericidal or bacteriostatic depending on the ratio MBC/CMI. If MBC/MIC≤ 4, the effect is bactericidal and when MBC/MIC>4, it's bacteriostatic (Harchaoui *et al.*, 2022). From the obtained ratio MBC/MIC, it can be noted that the crude and decoction extracts showed bactericidal effect against all bacterial strains tested. Contrarily, the aqueous extract of flowers exerts a bacteriostatic effect against *P. aeruginosa* and *M. luteus*.

4.3 Anti-inflammatory activities

4.3.1. Topical anti-inflammatory activity

Ear edema models induced by phlogistic agents have been extensively used as pharmacological tools for the investigation of new topical anti-inflammatory drugs, including natural products that are useful in the treatment of inflammatory skin disorders (Rodrigues *et al.*, 2016).

4.3.1.1. Effect on xylene-induced ear edema model

Results from this study revealed that the crude (CrE) and decoction extracts (DecE) of *E. arborea* at dose of 0.5 mg/ear exhibits substantial topical anti-inflammatory activity in the xylene-induced ear edema test as depicted in Figure 23. All extracts exerted significant (P<0.05) activity (0.5 mg/ear) against edematous response caused by xylene. Additionally, our findings indicated that CrE of L (leaves) and F (flowers) at a dose of 0.5 mg/ear reduces the edematous response by 83.53and 86.41 %, respectively. Whereas, DecE at same dose reduces the edematous response by 85.18% for L and 86.42% for F, respectively.

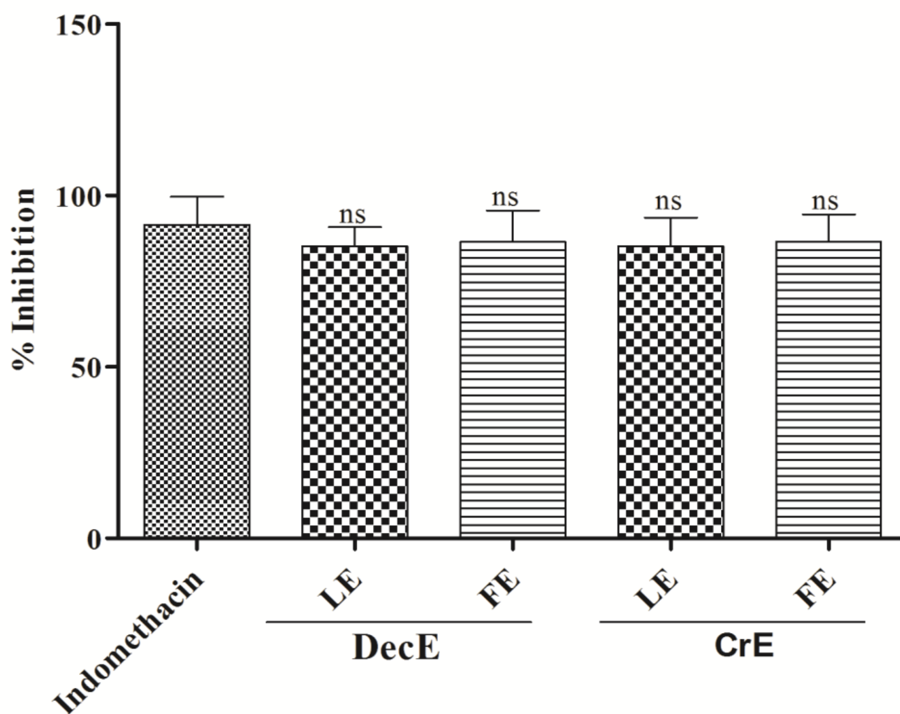

Figure 23. Topical anti-inflammatory effect of CrE and DecE of leaves and flowers on xylene-induced ear edema in mice. Data are presented as the mean ± SEM (n = 6), ns: no significant differences.

The topical application of xylene has been associated in inflammation, which is generates the increase of blood flow and edema formation and provoked by inflammatory eicosanoids, bradykinin,

serotonin and histamine (Rodrigues *et al.*, 2016). In addition, substance P and many neuropeptides released by these last inflammatory agents participate to the regulation of vasodilatation and plasma exudation by binding to the neurokinin-1 receptor (NK-1R) from sensory nerves and allowing by the release of inducible nitric oxide synthase (iNOS) (Càrdeno et al ., 2014). Indomethacin, the non-steroidal anti-inflammatory drugs (NSAIDs) and the cyclooxygenase (COX) pathway blocking, are frequently effective in experimental model and to be involved in medical practice, which is responsible for reducing the inflammation and reliving the pain by impeding of the prostaglandins production (Nakalembe *et al.*, 2019). Non- steroidal anti-inflammatory drugs has been effective against the inflammation induced by croton oil and xylene (Rodrigues *et al.*, 2016).

4.3.1.2. Effect on croton oil-induced ear edema model

The topical croton oil-induced ear edema test as depicted in Figure 24. CrE and DecE of all extracts exerted significant ($P<0.05$) activity (0.5 mg/ear) against edematous response caused by croton oil. The reduction of edema by croton oil in mice of LE (leaves extracts) ranged from 69.13to 83.95% and FE (flowers extracts) ranged from 52.67 to 81.48 %. In comparison with indomethacin 91.35 % (0.5 mg/per ear).

On the other hand, croton oil by induced ear edema application is a stimulating model of intense inflammatory process, useful to studying potential anti-inflammatory of natural compounds, could be most effective in the treatment of inflammatory pain. Firstly, the croton oil, comprises a mixture of lipids, which has 12-*O*-tetracanoilphorbol-13-acetate (TPA), an ester of phorbol, as main component, increase the levels of eicosanoids production, including leukotrienes and prostaglandins (PGE_2), which are synthesized by 5-lipooxygenase (5-LOX) and cyclooxygenase-2 (COX-2) enzymes, respectively.

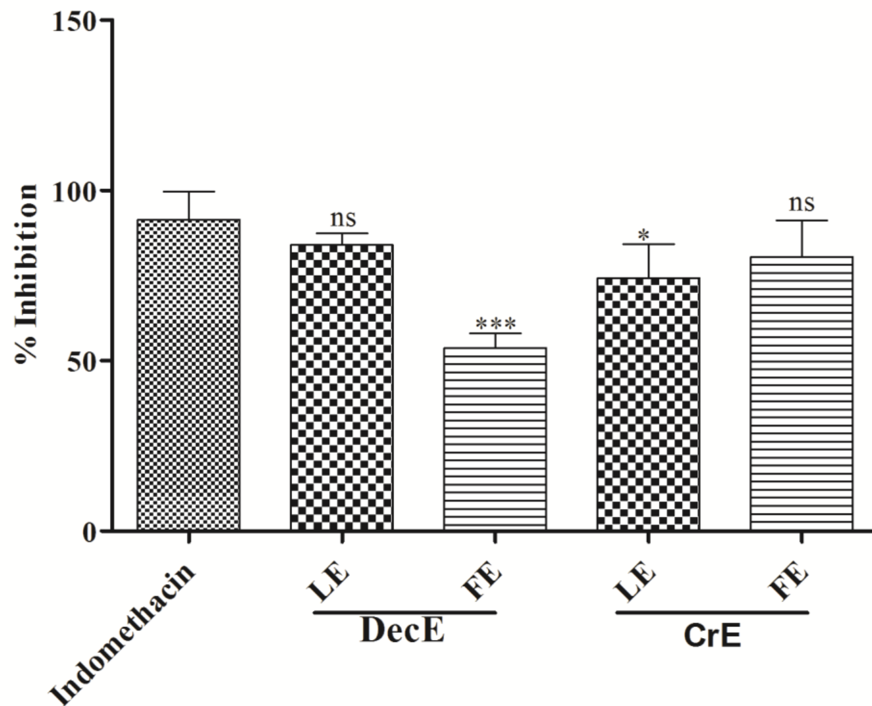

Figure 24. Topical anti-inflammatory effect of CrE and DecE of leaves and flowers on croton oil-induced ear edema in mice. Data are presented as the mean ± SEM (n = 6), ns: no significant difference, *: $p < 0.05$, ***: $p < 0.001$.

The inflammatory action of croton oil as judged also by increase vasodilatation, increase vascular permeability, inflammatory cells infiltration, leukocyte migration, release of histamine and serotonin and increase weight of ear mice (Da Silva *et al*., 2018; Abutaha *et al*., 2021). These different inflammatory mechanisms are in reply to dependent on the activation of pro-inflammatory cytokines, including TNF-α, interleukins, metalloproteinase and other mediators, such as phospholipase-A_2 (PLA_2), platelet activating factor (PAF) and arachidonic acid (AA). Therefore, the 12-o-tetracanoilphorbol-13-acetate (TPA), irritant constituent of croton oil is capable to active the protein kinase C (PKC) and the mitogen-activated protein kinase (MAPK), which promote the release of transcription factors, such as the nuclear factor κB (NF-κB) and the activating protein-1 (AP-1). Moreover, PKC and MAPK also stimulate the exudation and activation of a number of proinflammatory cytokines and mediators of inflammatory mechanisms (Rodrigues *et al*., 2016; Da Silva *et al*., 2018).

4.3.2. Oral anti-inflammatory activity

4.3.2.1.Effect on xylene-induced ear edema model

Results from this study revealed that the CrE from L and F at doses of 100–500 mg/kg exhibits substantial anti-inflammatory activity in the both xylene and croton oil-induced ear edema tests. Results are depicted in Figure 25. These extracts exerted significant dose-dependent activity (100, 250 and 500 mg/kg) and lowered the edematous response caused by xylene. Thus, our findings indicated that CrE from L and F at a dose of 500 mg/kg reduces the edematous response by 81.48 and 86.62 %, respectively. In xylene-induced ear edema assay, the standard drug, indomethacin (50 mg/kg), produced a strong anti-edematous effect in mice (91.64% inhibition).

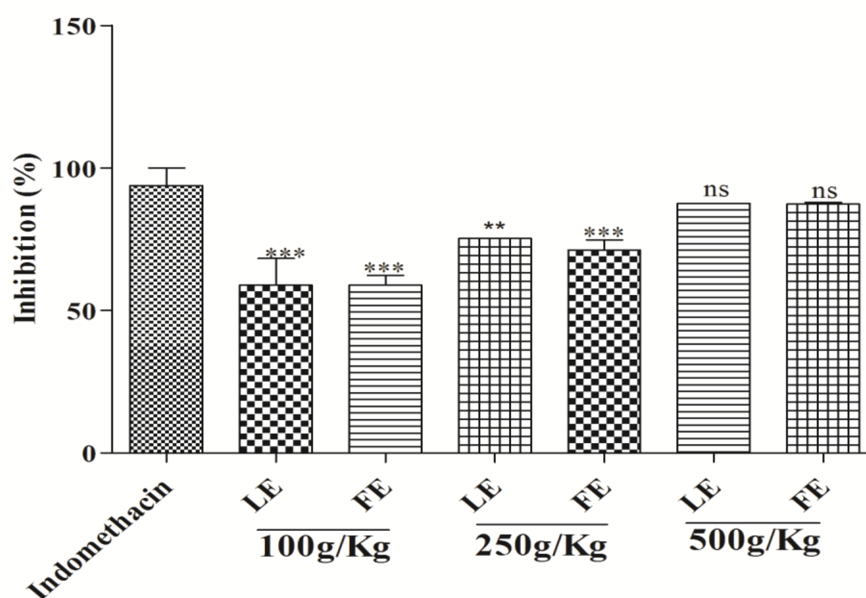

Figure 25: Oral anti-inflammatory effect of CrE of leaves and flowers on xylene-induced ear edema in mice. Data are presented as the mean ± SEM (n= ns: no significant difference, *: $p < 0.05$, ***: $p < 0.001$.

4.3.2.2. Effect on croton oil-induced ear edema model

The effect of CrE and DecE from L and F on croton oil induced ear edema in mice is shown in Table 15. The reduction of edema by croton oil in mice of CrE/DecE ranged from 20.00 to 87.70% for L and from 13.84 to 75.38% for F. In xylene-induced ear edema assay, the standard drug, indomethacin (50 mg/kg), produced a strong anti-edematous effect in mice (90.74 % inhibition).On the other hand, in croton oil induced ear edema assay the non-steroidal anti-inflammatory drug registered the edematous response by 91.64 % at same dose.

Table 15: Oral anti-inflammatory effect of CrE and DecE of leaves and flowers by xylene and croton oil-induced ear edema in mice. Data are presented as the mean ± SEM (n = 6), ns: no significant difference, **: $p < 0.01$, ***: $p < 0.001$.

Extracts/standard		Dose (mg/kg)	Inhibition(%)± SEM
			Croton oil
LE	CrE	100	$50.76 \pm 0.00^{***}$
		250	$63.08 \pm 0.00^{***}$
		500	87.70 ± 0.00^{ns}
	DecE	100	$20.00 \pm 8.70^{***}$
		250	$41.54 \pm 6.15^{***}$
		500	80.31 ± 6.74^{ns}
FE	CrE	100	$13.84 \pm 0.00^{***}$
		250	$50.77 \pm 0.00^{***}$
		500	$75.38 \pm 0.00^{**}$
	DecE	100	$38.46 \pm 0.00^{***}$
		250	$56.92 \pm 0.00^{***}$
		500	$75.38 \pm 0.00^{**}$
Indomethacin		50	91.64 ± 2.36

In this present study, the methanolic and decoction extracts of *E. arborea* possess significant in vivo topical and oral anti-inflammatory activities. We have evaluated the anti-inflammatory activities of the methanolic extracts of *E. arborea* to confirm the local use in folk medicine for inflammatory disorders revealed by the ethno botanical survey. Little review regarding *E. arborea* inflammatory effect is in line, which are no coherent with our current data regarding it anti-inflammatory potency on different experimental models tested *in vivo*. Akkol *et al*. (2007) displayed no remarkable anti-inflammatory effect of *Erica arborea* methanolic extract on topical administration using 12-*O*-tetradecanoyl-13-acetate (TPA)-induced ear edema model. Whereas, Amezouar *et al*. (2013) showed the anti-inflammatory protection with inhibition percentage value (59 %) at 400 mg/kg using carrageenan-induced hind paw edema model, this effect has been attributed by them to mainly flavonoids, major phenolic compound presents in *Erica arborea* (Amezouar *et al*., 2013).

According to the literature, the inflammatory responses inhibiting are generally attributed to the presence of plant-derived phenolic compounds (Sadowska *et al*., 2020; Kagambega *et al*., 2022). Many phytochemicals and phenolic compounds from plant extracts act in different mechanism ways during topical and oral application assays (Karbab *et al*., 2020; Karbab *et al*., 2021). Moreover, phenolic compound of *Theobroma cocoa* has shown in mouse with 12-O-tetracanoylphorbol-13-acetate (TPA) induced mouse skin, theirs potency to inhibit the expression of protein mitrogen-activated protein kinase (MAPK) and nuclear transcription factor Kappa β NF-kB factor (NFκβ)

(Villegas-aguilar et al., 2020). Thus, anthocyanins and hydroxytyrosol major phenolic compound of *Hibiscus sabdaariffa* and *Olea europea,* respectively showed anti-inflammatory effect, inhibiting the production of pro-inflammatory factors such as IL-6 and TNF-α (Sogo *et al.*, 2015; Yonezawa *et al.*, 2018).

In fact, the chemical nature of active substances of *E. arborea* may explain topical and oral anti-inflammatory effects.The biological activities of the extracts are closely related to their chemical composition (Bekkai *et al.*, 2022). Previous reports showed that oral administration of epicatechin results in anti-inflammatory effects. On the other hand, research findings indicated that catechin inhibits COX-1 and COX-2 expressed in inflammatory cells and other stimuli, and consequently causes decreased prostaglandin synthesis (Al-Sayed and Daim, 2018; Dias *et al.*, 2018). The capacity of epicatechin to inhibit degranulation of neutrophils was also shown in the study of Vilar and colleagues (Vilar *et al.*, 2016). Thus, kaempferol glycosides isolated from plants exhibited considerable anti-inflammatory effects, demonstrated by previous studies (Parveen *et al.*, 2007; Meloa *et al.*, 2009 ; Khan *et al.*, 2020) . In this respect, it was reported that flavonoids are known as inhibitors of TNF-α, IL-6 production, and iNOS expression (Parveen *et al.*, 2007; Peluso *et al.*, 2013) .

4.4. Analgesic activity

Results from the analgesic activity induced by acetic acid revealed that the crude extracts (CrE) of leaves (L) and flowers (F) exhibit a significant antinociceptive effect against acetic acid-induced abdominal constriction in mice in a dose-dependent fashion (100, 250 and 500). At the dose of 500 mg/kg, L and F extracts were significantly ($P<0.05$) reduced the abdominal constriction in mice with values of 89.34, 91.74 respectively, compared with aspirin as standard (Table 16).

Table 16: Analgesic effect of CrE of *E. arborea* L against acetic acid-induced abdominal contractions in mouse. Data are given as the mean ± SEM (n = 5), ns: no significant difference, ***: $p < 0.001$.

Extracts/standard	Dose (mg/kg)	Number of writhing	Inhibitory ratio (%)
Aspirin	100	10.00±4.58	83.49±3.56
LE	100	49.67±1.15***	18.04±1.90***
	250	10.00±1.00ns	83.49±1.65ns
	500	6.33±2.08ns	89.34±3.43ns
FE	100	41.00±1.00***	32.34±1.65***
	250	11.33±0.57ns	81.29±0.95ns
	500	4.67±1.15ns	92.29±1.90ns

The administration of subcutaneously acetic acid induced peripheral analgesic activity (Yimer *et al.*, 2020). Furthermore, it's responsible for the high release of free pain-causing substances such as arachidonic acid by cyclooxygenase and prostaglandin pathways, which has the face to sensitization of primary chemosensitive afferent nociceptors, thus making the visceral pain inside the treated peritoneal fluids via rising blood flow (Fyad *et al.*, 2020). Significant results acquired during this study, give us to note that the plant has a vigorous analgesic activity. However, *Erica arborea* is a plant rarely reported in scientific reports for its analgesic activities particularly remarked for its stem extracts and flower extracts. In the present study, the CrE of leaves and flowers exhibit a significant analgesic activity that may be linked either to the leadership of natural substances acting by blocking different inflammatory synthesis (Demsie *et al.*, 2019).

The increase in analgesic activity with increasing doses of the extract might be attributed with an increase in concentration of phytochemicals that possess analgesic activity with the maximum dose (Yimer *et al.*, 2020).The currently available standard drugs for pain remain the mainstay for managing and treating these disorders (Tamrat *et al.*, 2017; Yimer *et al.*, 2020). It is well established that NSAID relieve the pain response peripherally by inhibiting production of prostaglandins, thromboxane, and other inflammatory mediators by acting on cyclooxygenase enzymes (Subedi *et al.*, 2016). For this point of view, phenolic compounds from medicinal plants, which are found in methanolic extracts of *E. arborea* have been reported to possess a significant analgesic activity, especially in prostaglandins pathway inhibitions (Subedi *et al.*, 2016). The analgesic activity of the methanolic extracts confirm the traditional medicine use of *Erica arborea* in pain.

4.5. Anti-urolithic activities

This present study involved both CaOx nucleation and crystal agglomeration, as two important processes in the urinary tract for crystal retention (Ly *et al*., 2021). The results shown in (Table 17), given indication for different methanolic extracts from *E .arborea* at doses of 62.5–500 µg/mL, which have significant tremendous anti-urolithiatic effects for CaOx formation and also promote dose-dependent manner in vitro tests. In which, at highest concentration of 500 µg/mL, the methanolic flower extract had the larger percentage of inhibition against calcium oxalate nucleation compared to other extracts, with value of 98.66± 0.53 ($p < 0.05$), which was higher than allopurinol at value of 97.85 ± 1.26. Thus, the methanolic leaf extract, had the best potency on inhibitory activity with value of 75.63 ± 0.97 ($p < 0.05$) against crystals aggregation compared to other extracts, while positive control, allopurinol was nearly similar to methanolic leaf extract presented percentage of inhibition at 85.74 ± 0.16.

The wide use of herbal plants in the treatment of kidney stones and development of antiurolithiasis drug, are largely expanded in the world, which are presented minimal side effects (Salem *et al*., 2020; Kumar *et al*., 2021). The curative effect and efficiency of medicinal plant in anti-crystallization properties may be associated with several chemical phytoconstituents can act as inhibitors, that are presents in the extract and so to the method of extraction. (Salem et al., 2020; Mammate *et al*., 2022). Considering the ethnopharmacological data indicating traditional utilization of *E. arborea* against kidney stones, there has been no scientific documentation established on antiurolithic activity of leaves and flowers extracts of *E. arborea*. Besides, this herbal plant is probably auspicious for the inhibition and dissolution of kidney stones and using for the complementary treatment of individual suffering from kidney stones.

The results of previous researches have shown that the flavonoids-rich plant extracts could effectively provide the anti-urolithiasis activities correlating with their anti-inflammatory, antibacterial properties and other effects (Zeng and Jiang, 2019).

Table 17. Anti-urolithiatic effect of extracts evaluated by nucleation and aggregation assays. LE: leaves extract, FE: flowers extract. Data are presented as the mean ± SD (n=3), ns: no significant difference, *: $P < 0.05$.

Extracts/standard	Dose (µg/mL)	Inhibition (%) ± SD	
		Nucleation	Aggregation
LE	62.5	$88.45 \pm 0.89^{*}$	58.56 ± 4.79^{ns}
	125	$91.78 \pm 1.06^{*}$	72.93 ± 1.83^{ns}
	500	96.49 ± 0.5^{ns}	75.63 ± 0.97^{ns}
FE	62.5	93.79 ± 4.59^{ns}	65.11 ± 0.24^{ns}
	125	96.17 ± 0.13^{ns}	70.17 ± 0.41^{ns}
	500	98.66 ± 0.24^{ns}	72.87 ± 0.00^{ns}
Allopurinol	62.5	96.09 ± 2.60	73.04 ± 2.52
	125	97.70 ± 0.97	78.16 ± 2.27
	500	97.85 ± 1.26	85.74 ± 0.16

Flavonoids contributed for treating the formation of urinary stones in kidney by dissolution potency of calcium oxalate crystal or prevention of CaOx super saturation (Ly *et al.*, 2021). It is largely indicated in literature that flavonoids, tannins, coumarins, terpenoids isolated from different antiurolithiatic plant can lead to diuretic and anti-urolithiatic properties (Ahmed *et al.*, 2018; Gupta and Kanwar, 2018).

4.6. Acute toxicity

For the acute oral toxicity evaluation, both morbidity and mortality were monitored for 48 h periods of 14 days. Our findings indicate that methanolic extracts of *E. arborea* does not show any behavioral changes in treated mice throughout the examination period. No death, symptoms, and signs of toxicity were recorded in any of the treated animals during the fourteen days of experimentation. The lethal dose (LD_{50}) was higher than 5 g/kg BW for mice. Similarly, our findings agree with those of Amroun *et al.* (2021), who demonstrated that the aerial part aqueous extract of *E. arborea* is non-toxic even at a dose of 5 g/kg in the acute toxicity study.

CONCLUSION

CONCLUSION

Since very old times, plants have been generated much attention and used in the folk medicine by several indigenous herbal practices in human health to cure several diseases because their nontoxic implications and biological activities such as antioxidant, antibacterial, anti-inflammatory, analgesic and anti-urolithiatic. *Erica arborea*, a plant grows in the forests of the Mediterranean basin, is widely distributed in Algeria. Its aerial parts are mainly used by the local Algerian population for their antibacterial, anti-inflammatory action and kidney stone diseases.

In the first point, an ethnomedicinal data revealed that *Erica arborea*. L plant was the major plant used in the ethnobotanical practice. Additionnally, the extraction of phenolic compounds was carried out by different methods and their yield of extraction could be influenced by several factors include the method of extraction, the part used, the nature of extracted phytochemical compound and polarity of the solvents used.

E. arborea L. contains various compounds such as polyphenols, flavonoids, hydrolyzable tannins, quinones, anthraquinones, saponins and terpenoids in both leaves and flowers extracts. All the tested extracts of leaves (LE) and flowers (FE) showed an appreciable total content of phenolic compounds, flavonoids and condensed tannins. The highest total phenolic and flavonoids contents was present in the ethyl acetate extracts (EaEs) of leaves and flowers ranging from 649.38 to 944.55 (µg gallic acid equivalent/mg dry extract) and 65.31 to 67.15 (µg quercetin equivalent/mg dry extract), respectively. While, the highest condensed tannins content was present in the crude extract (CrE) of leaves and aqueous extract (AqE) of flowers with values of 280.37 ± 1.65 and 337.88 ± 1.88 (µg catechin equivalent/mg dry extract) . In a similar fashion, HPLC-MS analysis of hydromethanolic extracts (leaves, flowers) revealed also the presence of three known compounds identified as epicatechin, palmitic acid, and kaempferol-3-*O*-glucoside.

The ethyl acetate extract (EaE) of flowers and the crude extract (CrE) of leaves exhibited the better antioxidant activity by 2,2-diphenyl-l-picrylhydrazyl (DPPH) scavenging test (IC_{50} =17.72 µg/mL) and reducing power test (IC_{50} = 0.29 µg/mL), respectively.

Furthermore, results showed that the extracts exhibited an antibacterial power at variable degrees against all the pathogens. The *E. arborea L.* flower extract showed maximum antibacterial activity

especially against *P. aeruginosa* and *M. luteus* with diameter of inhibition zones of 17.04 and 16.30 mm, respectively.

Moreover, the CrE and DecE of leaves and flowers caused a considerable anti-edematogenic effect in the xylene and croton oil-induced ear edema in mice; this effect was dose-dependent in two experimental models. CrEs significantly reduced abdominal contractions at a dose mg/kg500 in the acetic acid-induced writhing model and exhibited also significant anti-urolithiatic activity in nucleation and aggregation assays.

Finally, finding from the *in vivo* toxicological assay showed that CrEs of leaves and flowers are safe, where no deaths or changes in the behavior of treated mice even at 5g/kg in mice. Findings from this investigation suggest that leaves and flowers extracts of *E. arborea* L could be used as prominent sources of natural anti-urolithiatic, anti-inflammatory, antibacterial, antioxidant and analgesic agents. Also, our findings provided the utilization of *E. arborea L (leaves, flowers)* in traditional medicine. In conclusion, the field of this study needs to be completed by other supplementary experiments especially:

- Evaluate the antioxidant activity and anti-urolithiatic *in-vivo*
- Other *in vivo* models of anti-inflammatory and analgesic assays are needed.
- Isolation, purification and identification of potent phytochemical compounds responsible of different biological activities and further study of their molecular mechanisms underlying the pharmacological profile of these plant extracts.
- Assess other biological activities such as cytotoxic, antipyretic, diuretic, antiulcer...etc

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Appendices

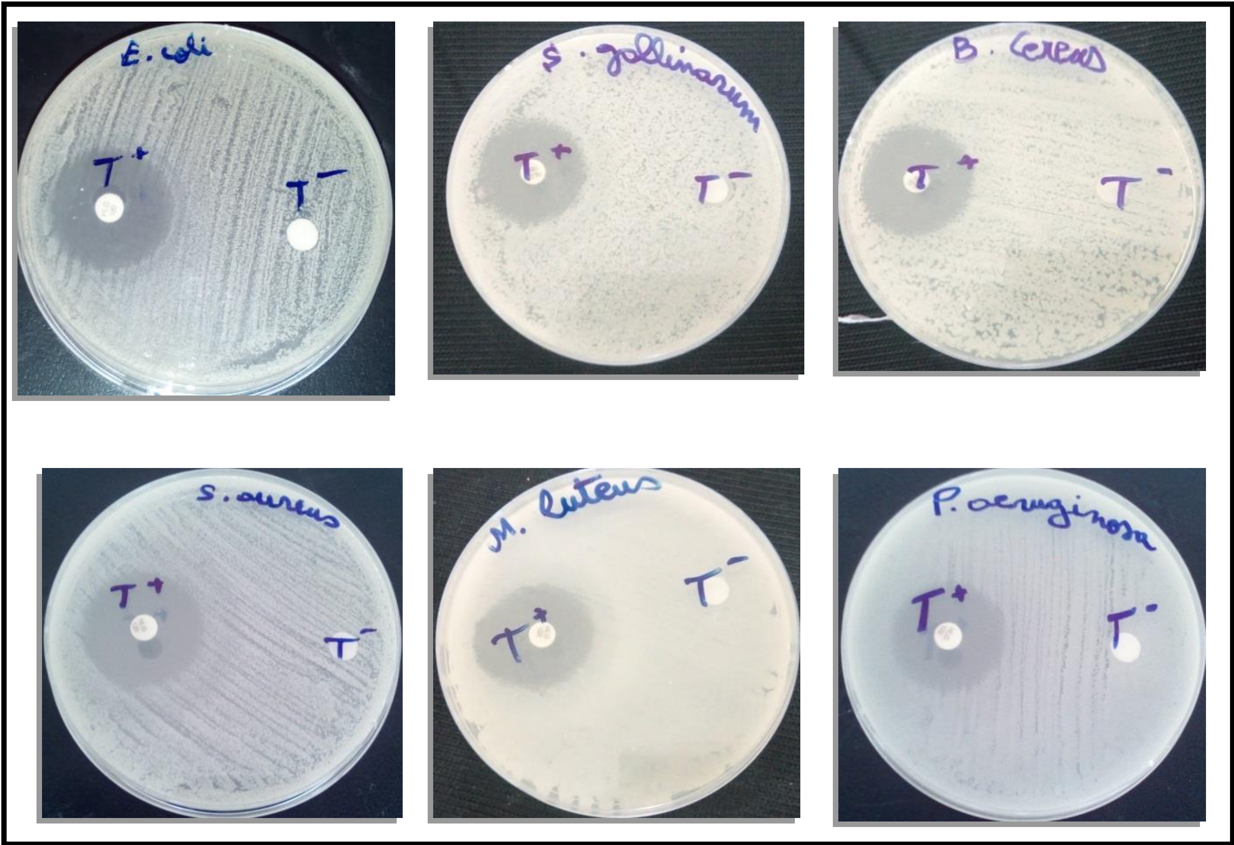

Figure 26: Determination of the zone of inhibition of the antibiotic against six bacterial strains

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Fractionation, Phytochemical Screening and Antioxidant Activity of Different Sub-Fractions from Leaves and Flowers of *Erica arborea* L.

Salima Amari[1,a], Ahlem Karbab[1,b,*], Lekhmici Arrar[1,c], Noureddine Charef[1,d,*]

[1]*Department of Biochemistry, Faculty of Natural and Life Sciences, Ferhat Abbas University of Setif-1, 19000 Algeria*
[*]*Corresponding author*

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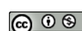
ABSTRACT

The purpose of this study was to prepare eight sub-fractions from leaves and flowers of *Erica arborea* L., characterize their phytochemicals constituents and investigate their potential antioxidant, in order to validate the beneficial medicinal properties of this shrub in Algeria folk medicine. Total polyphenols, flavonoids and condensed tannins contents were determined using Folin-Ciocalteu's, aluminum chloride and vanillin reagents, respectively. The *in vitro* antioxidant activity was evaluated by using 2,2-diphenyl-l-picrylhydrazyl and reducing power assay. *E. arborea* L. contains various compounds such as polyphenols, flavonoids, tannins, quinones, anthraquinones, saponins and terpenoids in different sub-fractions. All the tested extracts showed an appreciable total phenolic, flavonoids and condensed tannins contents as well as strong antioxidant capacity. The highest total phenolic and flavonoids content was found in the ethyl acetate extracts ranging from 649.38 to 944.55 mg gallic acid equivalent/g dry extract and 65.31 to 67.15 mg quercetin equivalent/g dry extract, respectively. Whereas, the highest condensed tannins content was found in the crude extract for leaves and aqueous extracts for flowers. The ethyl acetate extract of the flowers and the crude extract of leaves exhibited the better antioxidant activity by DPPH assay (IC_{50} =17.72 µg/mL) and reducing power assays (IC_{50} = 2.91 µg/mL), respectively. Our findings indicate that leaves and flowers extracts are rich in natural antioxidant substances and have good qualities in antioxidant properties and may be beneficial against diver's disorders related to free radicals.

[a] s.amari@centre-univ-mila.dz https://orcid.org/0009-0003-1860-2229
[b] ahlem.karbab@univ-setif.dz https://orcid.org/0000-0002-6036-1428
[c] lekharrar@univ-setif.dz https://orcid.org/0000-0003-3822-1469
[d] charefnr@univ-setif.dz https://orcid.org/0000-0001-6730-578X



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Introduction

Free radicals are highly reactive molecules, promising to the initiation of multiple chain reactions with other oxidants particles, which contain one or more unpaired electrons involve some degree of oxidative stress (Köroğlu et al., 2018; Akgül et al., 2022; Krupodorova et al., 2022). When the chain reaction of these radicals takes place in a cell, it can cause damage or death to the cell (Selamoglu, 2018). The oxidative stress is the major leading cause of many pathological disorders including neurodegenerative and cardiovascular diseases (Selamoglu et al., 2020). Many of synthetic antioxidants were used such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are casted to be responsible for some carcinogenesis problems and toxicological effect in liver (Chaouche et al., 2018; Kına et al., 2021). Since very old times, plants have been generated much attention and used in the folk medicine by several indigenous herbal practices in human health to cure several diseases because their nontoxic implications (Phuyal et al., 2020; Mohammed et al., 2022). Due to the use of various plant species as a resource of naturally occurring products, the study of their antioxidant ability and biological activities augmented in recent years (Selamoglu et al., 2017; Chaves et al., 2020; Phuyal et al., 2020, Karbab et al., 2021; Pehlivan et al., 2021). Thus, these studies could contribute to establishing the value of these species as a source of new antioxidant drugs (Chaves et al., 2012; Sevindik et al., 2017). Antioxidants are tremendously important substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress (Almada-Taylor et al., 2018; Unal et al., 2022). Phenols and flavonoids form different origin include fruits, vegetables, and medicinal and aromatic plants, are phytoconstituents gaining reputation for their antioxidant abilities (Karbab et al., 2019; Phuyal et al., 2020; Uysal et al., 2021). These components are well known for a variety of therapeutic properties on health of human (Selamoglu, 2017a; Selamoglu, 2017b; Jiang et al., 2019; Selamoglu and

Akalin, 2019). The genus Erica (Ericaceae) contains more species spread all over the world, three of which are that found abundantly in the flora of Algeria (Guendouze et al., 2015). *Erica arborea* grows commonly in Algeria and its medicinal properties is exploited by it enter in herbal tea composition (Guendouze et al., 2015; Suna et al., 2018). This shrub is known for the treatment of several health benefits such as digestive disorders include constipation and have excellent diuretic, anti-inflammatory and anti-lithiatic importance (Suna et al., 2018; Amroun et al., 2021). Several phytocomponents such as polyphenols, flavonoids, alkaloids been described from different parts of *E. arborea* (Luis et al., 2011) and biological activities of these natural antioxidants are responsible for several antioxidant properties to prevent diseases by scavenging free radicals and delaying or preventing oxidation of biological molecules (Afsar et al., 2018). Some experiments have been carried out in *E. arborea* aerial parts or their leaves alone regarding their antioxidant properties (Ay et al., 2007; Nazemiyeh et al., 2008; Köroğlu et al. 2018; Amroun et al., 2021). However, only one study has investigated its antioxidant activities of their separated vegetal parts (Luis et al., 2011). Therefore, the comparative study from different parts of the plants is still insufficient and no work done the effect of solvents in extracting phytochemicals from the leaves and flowers of the plant. So, the main objective of this study was conducted to carry out a study of different solvent extracts (methanol, chloroform, ethyl acetate, aqueous extracts) prepared for separated vegetal parts (leaves and flowers) of Algeria *E. arborea* used in medicine as natural medicines, on their antioxidant capacities as well as on total phenolic, flavonoids condensed tannins content. The in vitro antioxidant capacity was performed by 2,2-diphenyl-1-picrilhidrazil (DPPH) radical scavenging and reducing power activity.

Materials and Methods

Plant Material

Different vegetal parts of *E. arborea* were collected from the mountain of Djebel of Tadergount, Bejaia, North of Algeria. The plant was identified by Pr H. Laouer (Laboratory of Valorization of Natural Biological Resources, University of Setif, Algeria) under voucher specimen (015/DBEV/UFA/19). The dried material was powdered and stored in darkness until use.

Bioactivity Guided Fractionation

The separated parts *E. arborea* were prepared by using solvents with different polarities (Karbab et al., 2020). Leaves and flowers was extracted by methanol using a ratio of 1:10 at room temperature, and then stirred during 24 hours. The methanolic extract was partitioned sequentially by fractionation with organic solvents (hexane, chloroform, ethyl acetate) in order of increasing polarity (Figure 1). All the four fractions of different vegetal parts were dried by evaporating respective solvent using rotary evaporator. All extracts were stored at 4°C till further analysis.

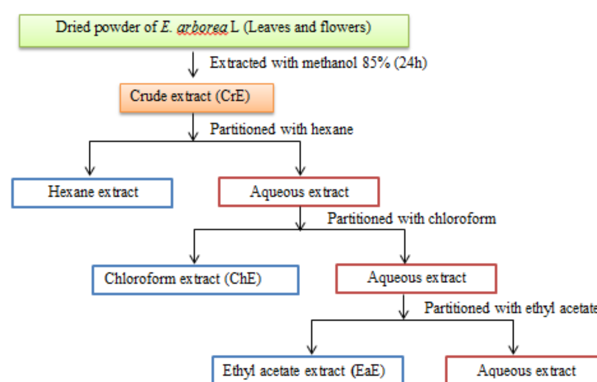

Figure 1 Preparation of different sub-fractions of *E. arborea* different parts. CrE: crude extract; ChE: chloroform extract; EaE: ethyl acetate extract.

Phytochemical Screening

Qualitative tests for the presence of different phytochemical compounds include: polyphenols, flavonoids, hydrolyzables tannins, free quinones, anthraquinones, anthocyanins, coumarins, alkaloids, terpenoids, and saponins according to published procedure (Karbab, 2020). These are qualitative analyses based on coloring and/or precipitation reactions.

Determination of Total Phenolic Flavonoid and Condensed Tannins Contents

The total phenolic contents in the different extracts were assessed by the Folin-Ciocalteau's method, in according to the method outlined by our previous publication (Karbab et al., 2020). Briefly, an aliquot of 100 µL of the extract was mixed with 500 µL of Folin-Ciocalteu's diluted reagent for 4 min, followed by the addition of 400 µL of a 7.5% Na_2CO_3 solution. After 1.5 h of incubation, the absorbance was measured at 765 nm. The total flavonoids content was determined by the colorimetric method described by (Karbab et al., 2020). One mL of each sample was added to 1 mL of aluminum chloride ($AlCl_3$) solution (2%). After 10 min of incubation, the absorbance of the mixture was measured at 430 nm. The condensed tannins in the extracts were determined by the vanillin method according to (Ali-rachedi et al., 2018). One mL of extract was mixed with 1.5 mL of 4% vanillin/methanol solution. Then, 750 µL of concentrated hydrochloric acid (HCl) was added. The mixture was allowed to stand in the dark at 20 °C for 20 min, and absorbance was determined at 500 nm. Different calibration curve was prepared. The polyphenol, flavonoids and condensed tannins content of the extract was expressed as µg Gallic Acid Equivalent/mg dry weight (DW), µg of Quercetin Equivalent/mg DW and µg Catechin Equivalent/mg DW, respectively. All determinations were done in triplicate (n = 3).

Antioxidant Capacity

DPPH scavenging assay

The antioxidant ability of extracts of *E. arborea* was performed by quenching DPPH (Charef et al., 2015). Briefly, freshly prepared DPPH solution (0.4 mM) was prepared in methanol, stored in an amber color bottle.

All the *E. arborea* extracts were dissolved in methanol and make various concentrations of an extract by applying serial dilution method. Add 1.25 mL of DPPH solution to 50µl of each serial dilution of extract. Additionally, 1.25 mL of DPPH solution is mixed with 50 µL of methanol as control without extract. The mixture was mixed well and incubated in darkness for 30 min. The assay was performed in triplicate, and the mean absorbance was calculated and noted at 517 nm. The butylated hydroxytoluene (BHT) is used as positive control. The percent of inhibition of DPPH radical was calculated using the formula: Radical scavenging activity (%) = $[(A_c - A_s)/A_c]\times100$; where A_c: the absorbance of the solution except the tested sample and A_s: the absorbance of extract or standard.

Reducing power assay

The reducing power assay was performed to estimate the ability of various extracts to reduce Fe^{+3} to Fe^{+2} (Bouaziz et al., 2015), with some modifications. According to this procedure, an aliquot of 400 µL of extract was mixed with an identical volume of both phosphate buffer (0.2 M, pH= 6.6) and potassium ferricyanide (1%). This mixture was then incubated for 20 min at 50°C in a water bath. The reaction was terminated by adding 400 µL of trichloroacetic acid (TCA) (10%), and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (400 µL) was added to distilled water (400 µL) and 80 µL of 0.1% ferric acid. The color intensity of the mixture was measured at 700 nm after 10 min of incubation. Reducing power assay (%) = $[(A_c - A_s)/A_c]\times100$; where A_c: the absorbance of the solution except the tested sample and A_s: the absorbance of extract or standard.

Statistical Analysis

Statistical analysis was performed by using the Graph Pad Prism (version 5.03 for Windows). In this study, statistical analysis was analyzed by one-way analysis of ANOVA. All determinations were carried in triplicate, and all results were estimated as the mean ± standard deviation (SD). Tests of significant differences were determined by multiple range tests at $P<0.05$.

Results

Extraction Yield

The yields of various extracts are shown in Table 1. The highest yield was noted with crude extract (CrE) in all used parts compared to other extraction by various solvents. Their yields decreased in the following order: CrE> AqE > EaE> ChE in *E. arborea;* which ranged from 21.28 to 1.2% for flowers, 21.21 to 1.36% for leaves Auxiliary, it was observed that the CrE extract (CrE) of flowers and leaves produced an almost similar maximum yield of phytochemicals about 21.28 and 21.21%, respectively. Although, chloroform extracts (ChE) of leaves, flowers displayed a lower yields with range from 1.2 to 1.36%. Color and consistency of extract varied also according to the extraction solvents, a mirrored powdery are recorded for Crude (CrE), ethyl acetate (EaE) and aqueous extracts (AqE), while the chloroform extract (ChE) of leaves and flowers has a matte powdery appearance.

Phytochemical Screening

The preliminary results from the phytochemical study of the studied extracts are shown in Table 2. Phytochemical investigation of the *E. arborea* extracts obtained from different parts of *E. arborea* (leaves and flowers) reveals the presence of metabolites such as polyphenols, terpenoids and quinones in all solvents extract. Whereas, in all the extracts, anthocyanins, coumarins, and alkaloids substances were found to be absents. The flavonoids, hydrolyzable tannins, anthraquinones and saponins were not found in all sub-fractions.

Total Phenolic Content, Flavonoids and Condensed Tannins Determination

The amounts of total phenolic (TPC), flavonoids (TFC) and condensed tannins (TC) were detected in tested extracts of all part of *E. arborea* and their results are given in Table 3. Firstly, in all sub-fractions, the highest level of polyphenols compounds were recorded in leaves (LE) followed by flowers (FE), which ranged from 80.45 µg GAE/mg dry extract for chloroform of FE to 944.55 µg GAE/mg extract for ethyl acetate of LE. The TPC decrease in the following order: EaE > CrE > AqE > ChE for LE and EaE>AqE > CrE> ChE for FE. Secondly, the flavonoids content are varying from 6.02 µg QE/mg extract for aqueous of FE to 67.15 µg QE/mg extract for ethyl acetate of LE. The largest amount of flavonoids was obtained via LE followed by FE. The TFC decrease in the following order: EaE> CrE> ChE> AqE for LE and FE. Furthermore, the condensed tannins content ranged from 14.77 (µg CE/mg extract) for chloroform of FE to 337.53 (µg CE/mg extract) for crude of LE. The TC decrease in the following order CrE> AqE >EaE > ChE for LE and AqE > CrE > EaE > ChE for FE.

Antioxidant Capacity

DPPH scavenging assay

The results revealed that all extracts scavenged the DPPH radical with an IC_{50} values varying from 38.18 to 60.16 µg/mL for leaves and from 17.72 to 65.29 µg/mL for flowers. All these values are higher than synthetic drug BHT with an IC_{50} value 87.65 µg/mL. The highest DPPH radical scavenging activity was exhibited with EaE of flowers with IC_{50} values of 17.72± 0.00 µg/mL, following by CrE of flowers with IC_{50} values of 24.81± 0.00 µg/mL, respectively. The lowest value was obtained by ChE of leaves.

Table 1. Yield, color and consistency of *E. arborea* extracts.

Extracts	Extraction yield (%)		Color and Consistency	
	LE	FE	LE	FE
CrE	21.21	21.28	Dark brownish mirrored powder	Dark brownish mirrored powder
ChE	1.36	1.2	Dark greenish mate powder	Clair greenish mate powder
EaE	6.84	5.32	Clair orangish mirrored powder	Clair orangish mirrored powder
AqE	15.81	12.20	Dark brownish mirrored powder	Dark brownish mirrored powder

CrE: crud extract; ChE: chloroform extract; EaE: ethyl acetate extract; LE: leaves extracts; FE: flowers extracts.

Table 2. Phytochemical screening of different extracts from *E. arborea* parts

Phytochemicals		Extracts			
		CrE	ChE	EaE	AqE
Polyphenols	LE	+++	+	+++	++
	FE	+++	+	++	++
Flavonoids	LE	++	-	+++	++
	FE	++	-	+++	-
Hydrolysable tannins	LE	+++	-	-	+++
	FE	+++	-	-	+++
Saponins	LE	-	+++	-	++
	FE	-	+++	-	+
Anthraquinones	LE	+	-	+++	+
	FE	+	-	+++	+
Quinones	LE	++	+	++	++
	FE	++	+	++	++
Coumarins	LE	-	-	-	-
	FE	-	-	-	-
Anthocyanins	LE	-	-	-	-
	FE	-	-	-	-
Terpenoids	LE	++	++	++	+
	FE	++	++	++	+
Alkaloids	LE	-	-	-	-
	FE	-	-	-	-

Key: += less presence, ++ =middle presence, +++=fort presence, -=absence. CrE: crud extract; ChE: chloroform extract; EaE: ethyl acetate extract; LE: leaves extracts; FE: flowers extracts.

Table 3 Total polyphenols, flavonoids and condensed tannins of various part extracts of *E. arborea*. The data represent the mean ± SD of three determinants.

Extracts		TPC (µg GAE/mg dry extract	TFC (µg QE/mg dry extract	TC (µg CE/mg dry extract
CrE	LE	591.58 ± 0.97	51.12 ± 1.42	337.53 ± 1.88
	FE	416.07 ± 1.46	25.25 ± 1.68	212.36 ± 0.23
ChE	LE	109.24 ± 0.49	18.53 ± 0.32	30.60 ± 1.41
	FE	80.45 ± 0.24	14.58 ± 1.99	14.77 ± 0.23
EaE	LE	944.55 ± 1.95	67.15 ± 0.04	173.53 ± 0.47
	FE	649.38 ± 1.95	65.31 ± 0.56	267.12 ± 1.06
AqE	LE	489.86 ± 1.46	9.37 ± 0.04	262.62 ± 0.12
	FE	481.24 ± 1.95	6.02 ± 0.11	280.37 ± 1.65

CrE: crude extract; ChE: chloroform extract; EaE: ethyl acetate extract; LE: leaves extracts; FE: flowers extracts.

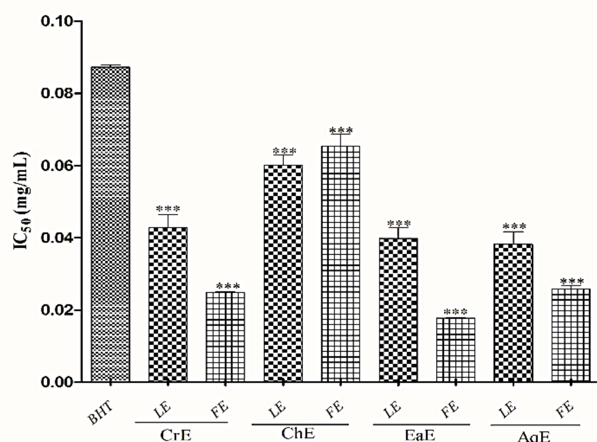

Figure1 DPPH radical scavenging activity of leaves, flowers and stems extracts of *E. arborea*.

CrE: crude extract; ChE: chloroform extract; EaE: ethyl acetate extract; LE: leaves extracts; FE: flowers extracts. Data are presented as IC_{50} values. Each value represents the mean ± SD (n = 3). ***: P≤ 0.01.

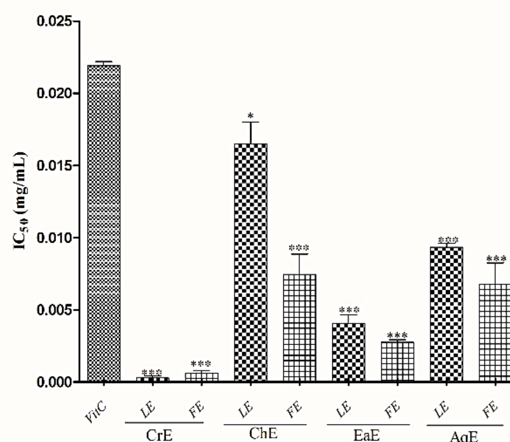

Figure 2 Reducing power of leaves, flowers and stems extracts of *E. arborea*.

CrE: crude extract; ChE: chloroform extract; EaE: ethyl acetate extract; LE: leaves extracts; FE: flowers extracts. Data are presented as IC_{50} values. Each value represents the mean ± SD (n = 3). ns: no significant difference, *: P≤ 0.05, ***:P≤ 0.001.

Reducing power assay

In the current test, the reducing power ability was determined for all extracts of *E. arborea* (Figure 2). All the extracts except chloroform extract displayed good reducing power by the reduction of Fe^{3+}/ferricyanide to Fe^{2+}-ferrozine complex by electron donation capacity. The reducing power of the leaves is ranged from values of 2.91 to 151.21 µg/mL, whereas, the IC_{50} of flowers is ranged from values of 6.22 to 74.71 µg/mL. In comparison to ascorbic acid, the greatest reducing antioxidant power was recorded for CrE of leaves, followed by CrE of flowers with an IC_{50} = 2.91 µg/mL and 6.22 µg/mL, respectively. Whereas, ChE of leaves displayed weak chelating properties. The trend of the total reducing power of the all extracts was; CrE> EaE > AqE > ChE >.

Discussions

Extraction is the first step for polyphenolic analysis, which consists in their isolation from plant materials (Chaouche et al., 2018). The variability of extraction yield depends on the plant part used as well as the solvent used. These results are in agreement with those obtained by Luis et al. (2011), who shown that the crude of *E. arborea* from the LE and FE recovered a better yield (Luis et al., 2011). Extraction yield of bioactive compounds from plant materials depends on the polarity of the extracting solvent (Do et al., 2013; Masoko, 2017; Chaouche et al., 2018). The difference in polarities of the extraction solvents might influence the nature of phytochemicals extracted in a sample (Dewi et al., 2020).

In the context to gain preliminary knowledge on the nature of chemicals compounds presents in various extracts of *E. arborea* systematic phytochemical investigation is accomplished. In the present study, polyphenols, hydrolyzable tannins, terpenoids, quinones, and anthraquinones detected in *E. arborea* of LE were in agreement with those obtained by Amezouar et al. (2013) from the dried leaves of *E. arborea* in which the presence of saponins was also reported. Flavonoids and phenolics are the main compounds isolated from Ericaceae species and that also contains terpenoids (Guendouze et al., 2015). Polyphenols, flavonoids, Tannins, saponins, quinones, anthraquinones, and terpenoids are widely known for their essential medicinal properties (Jiang et al., 2019).

The content of phenolic, flavonoids and condensed tannins was largely influenced by extraction solvents and its polarity, as well as by the vegetal parts. The quantitative determination these compounds are widespread assays (Luis et al., 2011). Firstly, some auteurs investigated the phenolic content of *Erica arborea* in several parts of the shrub (Luis et al., 2011; Suna et al., 2018). In general, LE has the highest amount of phenolic compounds. This result is similar to those obtained by Lius et al (2011). Suna et al. (2018) studied methanol extract of dried leaves and found value at 749.48 ± 34.46 mg gallic acid equivalent/g extract) of total phenolic content. However, this amount is higher than found in crude extract of LE (591.58 ± 0.97mg GAE/g of dry weight). Luis et al. (2011) examined different parts of methanol extracts from *E. arborea* such as leaves and flowers for their phenolic contents with 260.2 ± 1.9 (mg gallic acid equivalent/g extract) for leaves and 178.1 ± 0.2 (mg gallic acid equivalent/g extract) for flowers. These values obtained by this study were lower to those of present study.

The EaE of *E. arborea* was found to have high content of total phenols especially in flowers extracts. These results are in a good agreement with the literature (Ay et al., 2007; Köroğlu et al. 2018) for aerial part of *E. arborea*. Additionally, Köroğlu et al. (2018) were conducted the study of several *Ericaceae* species of Turkish, and concluded that their richness on phenolic compounds, and that ethyl acetate is one of the best solvents for their extraction (Köroğlu et al. 2018). Maximum total phenolic content found in ethyl acetate extracts of aerial part of *E. arborea* was 315.52±3.81 (µg pyrocatechol equivalents/mg extract) and 875.5 (mg gallic acid equivalent/g extract) in previous studies of Ay et al. (2007) and Köroğlu et al. (2018), respectively (Ay et al., 2007; Köroğlu et al. 2018). It was observed the effect of ethyl acetate solvent on TFC is similar to that on TPC which their content increased with solvent polarity; this is in agreement with the polar nature of flavonoids (Rebaya et al., 2015; Kohoume et al., 2017). Thus, Polar extract (EaE and CrE) showed more flavonoids than apolar extract (ChE) (Rebaya et al., 2014; Dewi et al., 2020).These results accorded to study of Dewi et al. (2020), which has the highest flavonoids content on ethyl acetate extract of *Scorodocarpus borneensis* Becc bark. Additionally, the ethyl acetate extract of mixed parts of *E. arborea* was found to be richest in terms of flavonoids (150.42±1.63 µg quercetin equivalents/mg extract) contents. Flavonoid content of leaves was higher than flowers. These results are similar to those reported by Rebaya et al. (2015). The distribution of condensed tannins across all the used parts using different solvents exhibited also a very great difference. There are no literature data for condensed tannins contents of leaves and flowers extract, but in this work they were shown to be rich in these compounds.

Regarding to the obtained phenolic, flavonoids and condensed tannins compounds values, it is difficult to establish a correct comparison between our results and those of literature data. The geographical and climatic conditions can lead to significant difference in both the concentration of bioactive compounds in plant and their bioactivity for human health (Guendouze et al., 2015). Medini et al. (2014) found that the flowering stage of plant *Limonium delicatulum* had a higher level of phenols compound than the vegetative stage (Medini et al., 2014). It was also known the phenolic content was affected by solvent polarity and a particular part of the plant (Dewi et al., 2020). This large difference in this content can be explained by other factors, among these the origin, the harvested period (Dewi et al., 2020). These amounts may be also affected by the presence of different amounts of other compounds (Aryal et al., 2019).

Due to the complex nature of phytochemicals and their interactions, the importance of using various methods based on different mechanisms for a comprehensive study of the antioxidant properties of plant extracts has been argued (Bekkai et al., 2022). The effect of extracts at different concentrations was studied for their ability of hydrogen or electron transferring ability measured using DPPH and FRAPS tests. The estimation of reducing power by chelating of ferrous ion Fe (III) or the utilization of free DPPH reagent in antioxidant assay among other methods

are very suitable and widely used for determining the antioxidant potency of plant extracts (Chaouche et al., 2018; Aryal et al., 2019; Phuyal et al., 2020). The purple - colored DPPH radical is capable turning to the colored-yellow as well as transformed into DPPH-H when interacted with antioxidant components presents in extracts (Aryal et al., 2019; Trinh et al., 2020; Baliyen et al., 2022). The formation of yellow colorless, diphenylpicrylhydrazine in solution can be quantified spectrophotometrically, which DPPH radical showed a maximum absorption at 515-528 nm (Selamoglu et al., 2017; Karbab, 2020). The reduction ability of reductants or extracts under acidic conditions indicated by the transformation of Fe (III)/ferricyanide complex to it Fe (II)/ferrous colored form by giving away an electron. The yellow color test solution changes to green and blue depending on the can be monitored by measurement of blue color at 700 nm (Selamoglu et al., 2017**;** Aryal et al., 2019). Some researchers investigated the antioxidant capacity of *Erica arborea* (Amezouar et al., 2013; Suna et al., 2018) for dried leaves, (Ay et al., 2007; Guendouze et al., 2015; Köroğlu et al. 2018) for aerial parts and (Luis et al.,2011) for separated aerial parts.

All the examined extracts were able to reduce DPPH and ferrous ion Fe (III) by donation of a hydrogen atom or electron. The scavenging activity against DPPH of all extracts with different polarities of the aerial parts of *E. arborea* native to Turkey were investigated in study of Köroğlu et al. (2018) in the same following order obtained in our study: EaE> AqE> CrE> ChE. All extracts exhibited strong antioxidant activities except the chloroform extract (Köroğlu et al. 2018). In FRAP assay, CrE presents the highest result and the ChE presents the lowest one (Aadesariya et al., 2017). These results were in agreement with those found by Chaouche et al. (2018) on aerial part from *Teucrium polium* which reported that methanolic extract exhibited highest reducing power ability when compared to other extracts. In comparison to the previous study of Amezouar et al. (2013), which studied the leaves of *E. arborea* macerated by methanol, it IC_{50} value is lower than the present study (Amezouar et al., 2013). Also, another investigation on the dried leaves macerated by methanol showed higher IC_{50} about 154.73 ± 1.59 µg/mL (Suna et al., 2018).

Hence, the strong antioxidant activity of *E. arborea* extracts of different vegetal parts could be due to their richness on natural antioxidant substances (Luis et al., 201; Köroğlu et al., 2018). Shrubs of the family of Ericaceae are known as natural sources of bioactive compounds associated to their phenolic compounds composition (Bekkai et al., 2022; Köroğlu et al., 2018). The high phenolic content found in these species is thought to be linked to their strong free-radical scavenging effects and potential health related to therapeutic functions (Ay et al., 2007; Marquez- Garcia et al., 2009; Guendouze et al., 2015; Köroğlu et al. 2018). Some researchers reported that there is a strong correlation between total phenolic contents and the antiradical effectiveness of extracts (Köroğlu et al., 2018; Hmaidosh et al., 2020). This correlation is consistent with the current study, particularly in the EaE and ChE for polyphenols content. This following trend shown in the concentrations of condensed tannins of the leaves extracts. This may imply that the reducing power of leaves had a direct relationship with the concentration of condensed tannins they contain.

The data obtained from this study reveal that EaE of flowers exhibited the most powerful antiradical effect than that of other extracts in different solvent extraction. This may be suggested by the kind of antioxidant phytochemicals such as flavonoid and phenolic compound present in these sub-fraction as well as the presence of other natural products. Also, these same extracts marked high amount of total phenols and flavonoids (Table1). Acoording to Sannigrahi et al. (2008), the low IC_{50} value of ethyl acetate fraction of *Enhydra fluctuans* Lour is due to presence of high polyphenolics and flavonoids (Sannigrahi et al., 2008). Moreover, in the study of Ay et al. (2007), the ethyl acetate extract of mixed parts (leaves and flowers) of *E. arborea* showed the highest phenolic compounds and antioxidant capacity than other extracts in the DPPH assay even higher than BHT, used as reference compound (Ay et al., 2007). In other study, the ethanolic extract of *E. arborea* exhibited notable antioxidant activity in FRAP and DPPH free radical scavenging with the high total polyphenols and tannins values (Pavlovic et al., 2009). Although, the effect of the antioxidant activity of the samples differs rendering of the nature of the solvent used which could be due to different antioxidant compounds extractable (Chaouche et al., 2018; Köroğlu et al. 2018).

In recent years the powerful antioxidant capacity of the flavonoids has been attracting much attention (Köroğlu et al. 2018). The ethyl acetate extract of aerial parts of *E. arborea* was found to be richest in terms of phenolic especially flavonoids contents which exhibited the highest DPPH antioxidant activity (Ay et al., 2007; Köroğlu et al. 2018). Furthermore, in plates sprayed with 1% vanillin-H_2SO_4 analysis detected mainly flavonoids such as kaempferol and luteolin in ethyl acetate extracts of aerial part of *Erica* species (Köroğlu et al. 2018). In aqueous extract among compounds found of aerial part of this shrub are as follow chlorogenic acid and five glycosylated flavonoids (Amroun et al., 2021). In previous study of Nazemiyeh et al. (2008) the methanol extract of the leaves of *E. arborea* afforded five flavonoids and exhibited a higher antioxidant activity than the propyl gallate used as standard (Nazemiyeh et al., 2008). Pavlović et al. explained the antioxidant capacity of *Erica* species, macerated in ethanol by the presence phenolic compounds and kaempferol-3-O-β-D-galactoside and quercetin (Pavlović et al., 2014). Flavonoids in the middle of natural phenolic compounds were known to possess the most potent radical-scavenging and to act in different mechanisms in the regulation of oxidative stress (Alrawaiq and Abdullah, 2014; Baliyen et al., 2022; Dewi et al., 2020; Masriani et al., 2020). Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Adaramola et al., 2017).

According to multiple reports in the literature, the structural characteristics of polyphenols is certainly the most important parameter, for free radical scavenging and reducing power capacities (Guendouze et al., 2015; Chaouche et al., 2018; Aryal et al., 2019; Dewi et al., 2020; Phuyal et al., 2020)**.** Therefore**,** phenolics possess one or

more aromatic rings, and one or more hydroxyl groups that are disposed to donate a hydrogen atom or an electron to a free radical (Chaouche et al., 2018; Aryal et al., 2019). The antioxidant activity of flavonoids isolated by Nazemiyeh et al. (2008) is a consequence of the presence of the phenolic moieties in the structures (Nazemiyeh et al., 2008).it was demonstrated the particular substitution pattern of free groups on the flavonoid skeleton influenced the potential of antioxidant activity. Thus, the presence of hydroxyl and-carbonyl group in the favonoid skeleton resulted in high FRAP potential and the presence of 2,3-double bond in conjugation with the 4-oxo function in the C-ring resulted potent radical scavenging ability (Selamoglu, 2017a; Afsar et al., 2018).

Conclusions

The results obtained from this study suggested that leaves and flowers extracts exhibited good antioxidant properties and might be used advantageously as an antioxidant agents for the protection against oxidative stress related to various cell damages, demonstrating by two different methods, namely DPPH and reducing power. Results obtained in this study demonstrated that the potency of electron /or hydrogen donors of these extracts of *E. arborea* separated parts grown in Algeria varies with the type of solvent, the vegetal part and their content on antioxidant compounds such as phenolic, flavonoids and condensed tannins. Moreover, the results also indicated that ethyl acetate sub-fraction of flowers extract exhibited the highest DPPH scavenging when compared with other sub-fractions of all vegetal parts used and also the crude extract of leaves exhibited the highest reducing properties. The results also showed correlation between phenolic, flavonoids and condensed tannins content and the antioxidant capacity of the various sub-fractions. Thereby, these results are preliminary and further testing of the activity of high sub-fractions and isolating procedures of the responsible antioxidants molecules of *E. arborea* would be interesting.

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Conflicts of Interest

Authors declare no conflict of interest.

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ملخص

الخلنج هي شجيرة صغيرة لها استعمالات تقليدية متعددة في منطقة بجاية بالجزائر حيث أنها معروفة بكونها مدرة للبول، تعالج الإمساك والتهابات المسالك البولية وحصوات الكلى والتهابات متعددة. إن هذه الدراسة تهدف إلى التحقق من فعالية الاستخدامات العلاجية للأجزاء المختلفة من النبتة بالإضافة إلى تقييم مختلف الأنشطة البيولوجية كالنشاطات المضادة للأكسدة، مضادات الجراثيم، مضادات الالتهاب (في الكائنات الحية)، التأثيرات المسكنة ومضادات حصوات المسالك البولية. أظهرت البيانات المأخوذة من الدراسة العرقية أن 28 ٪ من السكان المحليين الذين استخدموا هذه النبتة في الطب الشعبي. يحتوي الخلنج على مركبات كيميائية تم الكشف عنها في المستخلصات المختلفة للأوراق والزهور والتي تتمثل في البوليفينول والفلافونويد والتانينات القابلة للتحلل والكينون الحر والأنثراكينون والصابونين والتربينويدات. وبينت جميع مستخلصات الأوراق (LE)، ومستخلصات الأزهار (FE) وجود كميات معتبرة من البوليفينول والفلافونويد والعفص المكثف. كانت القيمة الإجمالية من مادة البوليفينول والفلافونويد في مستخلص أسيتات الإيثيل (EaEs) للأوراق والأزهار، والتي تراوحت من 649.38 إلى 944.55 ميكروغرام مكافئ حمض الغاليك / ملغ من المستخلص الجاف ومن 65.31 إلى 67.15 ميكروغرام من مكافئ كيرسيتين / ملغ من المادة الجافة، على الترتيب. بينما وجدت أعلى قيمة من العفص المكثف في المستخلص الخام للأوراق (CrE) والمستخلص المائي (AqE) للأزهار. كما أظهرت نتائج تحليل HPLC-MS أيضًا وجود epicatechin وpalmitic acid وkaempferol-3-*O*-glucoside في مختلف المستخلصات الميثانولية المائية المختبرة. و بين مستخلص الأسيتات ايثيل EaE للأزهار و المستخلص الخام CrE للأوراق نشاط ضد الأكسدة معتبر من خلال اختبار إزاحة جذر DPPH (IC_{50}= 17.72 ميكروغرام / مل) واختبار القدرة الارجاعية (IC_{50}0.29 ميكروغرام / مل)، على التوالي. أظهرت المستخلصات المختبرة فاعلية بدرجات مختلفة مضادة للجراثيم. وبالفعل مستخلص الأزهار أظهر نشاطًا جيدا مضادًا للجراثيم، خاصةً ضد *P. aeruginosa* و *M. luteus*. التقييم السمي بين أن (CrEs) التي تم تناولها عن طريق الفم لم تسبب الموت أو تغير في سلوك الفئران المعالجة. بالإضافة إلى ذلك، CrE و DecE بينت تأثير مضاد للتورم ضد وذمة الأذن الناتجة عن كزيلان أو زيت كروتون في الفئران. علاوة على ذلك تساهم CrEs في تقليل التقلصات البطنية بشكل كبير بجرعة 500 مغ / كغ في نموذج التلوي الناجم عن حمض الخل وأظهر أيضًا نشاطًا كبيرًا مضادًا لحصوات مجرى البول في فحوصات التنوي والتجميع. بالاضافة، تؤكد نتائجنا الاستخدام الطبي لهذه النبتة في الطب التقليدي وأن مستخلصات أوراق وزهور نبتة الخلنج يمكن استخدامها كموارد طبيعية مهمة كمضادات حصوات المسالك البولية، مضادات الالتهابات، مضادات الجراثيم، ومضادات الأكسدة ومسكنات.

الكلمات المفتاحية: الخلنج، دراسة عرقية، سمية، نشاطات بيولوجية، بوليفينول.

ABSTRACT

Erica arborea L. is a small shrub widely known in Algeria's region of Bejaia by its uses for diuretic purposes as well as to treat constipation, urinary tract infections, kidney stones and inflammation. This study aimed to investigate medicinal uses of the different plant parts of *E. arborea* and to examine their different biological activities such as antioxidant, antibacterial, anti-inflammatory (*in vivo*), analgesic and anti-urolithiatic. The data of an ethnopharmacological survey showed that 28 % of local habitants used this plant in folk medicine. *E. arborea* contains various compounds such as polyphenols, flavonoids, hydrolyzable tannins, quinones, anthraquinones, saponins and terpenoids in both leaves and flowers extracts (LE and FE), respectively. All the tested extracts showed an appreciable total content of phenolic compounds, flavonoids and condensed tannins. The highest total phenolic and flavonoids contents was present in the ethyl acetate extracts (EaEs) of leaves and flowers ranging from 649.38 to 944.55 µg gallic acid equivalent/mg dry extract and 65.31 to 67.15 (µg quercetin equivalent/mg dry extract), respectively. While, the highest condensed tannins content was present in the crude extract (CrE) of leaves and aqueous extract (AqE) of flowers. Results of HPLC-MS analysis also revealed the presence of epicatechin, palmitic acid, and kaempferol-3-*O*-glucoside in extracts. The EaE of flowers and CrE of leaves exhibited the better antioxidant activity by 2, 2-diphenyl-l-picrylhydrazyl (DPPH) scavenging test (IC_{50} =17.72 µg/mL) and reducing power test (IC_{50} = .029 µg/mL), respectively. Furthermore, results showed that the extracts exhibited an antibacterial power at variable degrees against all the pathogens. Indeed, the *E. arborea* flower extract showed maximum antibacterial activity especially against *P. aeruginosa* and *M. luteus*. The results of the toxicity assay showed that CrEs are safe, where no deaths or changes were noted in the behavior of treated mice. Moreover, CrE and decoction extract (DecE) caused a considerable anti-edematogenic effect in the xylene and croton oil-induced ear edema in mice dose-dependent in two experimental models. However, CrEs significantly reduced abdominal contractions at a dose mg/kg500 in the acetic acid-induced writhing model and exhibited also significant anti-urolithiatic activity in nucleation and aggregation assays. In addition, the results support the use of *E. arborea* in folk medicine and the leaves and flower extracts of *E. arborea* could be used as important resources of natural anti-urolithiatic, anti-inflammatory, antibacterial, and antioxidant and analgesic agents.

Keywords*:* *Erica arborea* L, ethnopharmacological survey, toxicity, biological activities, phenolic compounds.

Résumé

Erica arborea L. est un petit arbuste largement utilisé dans la région de Béjaia en Algérie à des fins diurétiques ainsi pour traiter la constipation, les infections urinaires, les calculs rénaux et les inflammations. Cette étude visait à étudier l'utilisation médicinale de différentes parties d'*E. arborea* et d'évaluer leurs différentes activités biologiques telles que antioxydantes, antibactériennes, anti-inflammatoires (*in vivo*), analgésiques et anti-urolithiatiques. Les données d'une enquête ethnopharmacologique ont montré que 28 % des habitants locaux utilisaient cette plante en médecine populaire. *E. arborea* contient des composés phytochimiques dans différents extraits testés de feuilles (LE) et fleurs (FE) tels que les polyphénols, les flavonoïdes, les tanins hydrolysables, les quinones, les anthraquinones, les saponines et les terpénoïdes. Tous les extraits testés ont montré des teneurs appréciables en polyphénols, flavonoïdes et tanins condensés. Les teneurs totaux en polyphenols et flavonoïdes les plus élevées ont été trouvées dans les extraits d'acétate d'éthyle (EaEs) de feuilles et fleurs, allant de 649.38 à 944.55 µg d'équivalent d'acide gallique/mg d'extrait sec et de 65.31 à 67.15 µg d'équivalent de quercétine/mg d'extrait sec, respectivement. Alors que la teneur en tanins condensés la plus élevée a été trouvé dans l'extrait brut de feuilles et l'extrait aqueux de fleurs. Les résultats de l'analyse HPLC-MS ont également révélé la présence d'épicatéchine, d'acide palmitique et de kaempférol-3-*O*-glucoside dans les extraits méthanoliques testés. L'extrait d'acétate d'éthyle (EaE) de fleurs et l'extrait brut de feuilles (CrE) ont montré le meilleure activité antioxydante par le test de piégeage du radical 2,2-diphényl-l-picrylhydrazyle (DPPH) (IC_{50} = 17.72 µg/mL) et le test de pouvoir réducteur (IC_{50} = 0.29 µg/mL), respectivement. Les extraits testés ont exhibé un pouvoir antibactérien variable vis-à-vis les agents pathogènes. En effet, l'extrait de fleur d'*E. arborea* a montré une meilleure activité antibactérienne, particulièrement vis-à-vis de *P. aeruginosa* et *M. luteus*. L'évaluation de la toxicité a montré que les extraits bruts (CrEs) administrés par la voie orale n'ont pas induit ni la mort ni le changement dans le comportement des souris traités. De plus, CrE et l'extrait de decoction (DecE) ont provoqué un effet anti-œdématogène considérable vis-à-vis de l'œdème de l'oreille induit par le xylène ou l'huile de croton chez les souris ; cet effet était dose-dépendant dans les deux modèles expérimentaux. De plus, les CrEs ont provoqué la réduction de manière significative les contractions abdominales à une dose mg/kg500 dans le modèle de contorsion induit par l'acide acétique et ont montré également une activité anti-urolithiatique significative dans les tests de nucléation et d'agrégation. De plus, nos résultats soutiennent l'utilisation d'*E. arborea* en médecine traditionnelle et les extraits de feuilles et fleurs d'*E. arborea* pourraient être utilisés comme des ressources importantes des agents naturels anti-urolithiatiques, anti-inflammatoires, antibactériens, antioxydants et analgésiques.

Mots clés *: Erica arborea* L, étude ethnopharmacologique, toxicité, activités biologiques, composés phénoliques.