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Chemical Constituents of *Capparis sinaica* Veill. Plant and its Antimicrobial Effects

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ABSTRACT

Capparis sinaica Veill. belongs to the family Capparaceae that has several uses in the Egyptian folk medicine for many years. Nothing could be traced about the chemical composition of the plant. Preliminary phytochemical screening of the plant, revealed the presence of tannins, flavonoids, carbohydrates and/or glycosides, resins, sterol, saponins and alkaloids. The highest concentration of total carbohydrates, nitrogen and protein were determined in the aerial part of the plant at winter, while the highest concentration of the total lipid content was determined at autumn. The highest concentration of total flavonoids, saponins and alkaloids were determined in the aerial part of the plant at winter, while the highest concentration of the total tannins content was determined in the root part of the plant at winter. Extraction, isolation and purification of the air-dried plant material using different chromatographic techniques provided five flavonoids and two alkaloids. Identification of the isolated compounds using different chemical and physical techniques (UV and 1H-NMR spectroscopy) allowed to characterize these compounds as quercetin, querctein-7-O-rutinoside, Luteolin, Kaempferol-3-galactoside and quercetin-7-glycoside as flavonoid compounds as well as Capparin A and Capparin B as alkaloid compounds. Extracts of ethyl acetate, methanol for aerial part of *Capparis sinaica* plant produced the best inhibition effects against the all tested fungi species, while each of hexane, benzene, chloroform and water of all fractions have not any anti-fungi activities against different fungi strains.

Key words: *Capparis sinaica*, Capparaceae, phytochemical, kaempferol, quercetin, Capparin

Introduction

The use of medicinal plants recently plays an important role in the search for new chemical bioactive agents. Their isolation from plant extracts resulted in a development of human diseases treatment and discovery of many useful drugs.

The Capparaceae composed of 45 genera and approximately 1000 species, distributed especially in the tropical and subtropical region, especially East Africa and South America. Plant of this family are annual, perennial some time climbers or tree (Duarte, 1995). The most plant of this family contains many active constituents such as alkaloids, tannins, saponins, steroids, terpenoids, flavonoids, phloatunnins, phenolic constituents and cardic glycosides. (Edeoga *et al*., 2005 and Daniel, 2005).Sama and Ajaiyeoba, (2006) stated that preliminary screening of both *Capparis thonningil* and *Capparis tomemtosa* revealed the presence of alkaloids, steroidal, saponine, tannins and cardiac glycoside.

Naturally grow of *Capparis* in various region is distributed in all the arid region. There are more than 250 species of the *Capparis*. Historically this plant has been used for cooking and medicinal purpose and its flower buds are treading in the international market and also its consummation. Due to this capability to grow in arid and semiarid region that are subject to extreme conditions. Therefore, it's important to encourage the studies and agriculture of this plant to resolved the problem related to its production (Cosge *et al*., 2005).

In the Arabian folk medicine, several *Capparis* species have many uses. The total herb of *Capparis sinaica* Veill. is used for bruises, childbirth, earache, headache, paralysis, snakebite and swelling. Also the fruits of *Capparis decidua* (Forssk.) Edgew are used to relieve difficult breathing as a laxative, anthelimintic and for nervous disorders. Furthermore, the leaves of *Capparis spinosa* are used for treating earache, coughs, expelling stomach worms and for diabetes (Shahina, 1994). While the bark is used for the treatment of gout, rheumatism and as a laxative and expectorant for chest diseases. The bark is also used in liver affections. Infusion of stem and root bark is used for diarrhea and febrifuge. Moreover, the flower buds and roots are used as renal disinfectants, diuretic, tonic and for arteriosclerosis and as compresses for the eyes (Batanouny, 1999).

Tuerkoez *et al.,* (1995) reported that, the leaves of *Capparis spinosa* and *Capparis ovata* are source of rutine and the amount of rutin from methanol extracts were 3.90 and 2.32%, in the first and second seasons respectively. Sharaf *et al*., (1997) the aerial parts of 3 *Capparis* species were investigated for their flavonoids constituents. Thirteeen flavonoids glycosides were isolated and identified. Giuffrida *et al*., (2007) reported that

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the buds of *Capparis spinosa* contained rutine and kaempferol-3-rutinoside which were the most abundant flavonol glucosides.

Sarragiotto *et al.,* (2004) stated that methanol extract of dried plant of *Capparis humilis*gave proline betaine, saccharose and 3-carboxy-N-methlpyridinlum which formed from saccharose and kaemferol3,7dirhmnoside which detected by Ali *et al*., (1992) isolated two new sperimidine alkaloids,14-N-acetylisocodonocarpine and 15-N-acetylcapparisine from the root bark of *Capparis deciduas*. Khanfar *et al*., (2003) isolated two new alkaloids, namely beta – sitosteryl glucoside-6\-octadecanoate and 3-methyl-2-butenyl-beta glucoside from *Capparis spinosa.* Su-Dongmin *et al*., (2007) isolated two new ^{1}H-indole-alkaloid glucosides, from root of *Capparist enera.* Hassan *et al*., (2007) studied the antimicrobial activity of the ethanolic extracts of *Capparis deciduas* root bark and found that all tested concentrations more potent than the standard and this fraction was active against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* but was inactive against *Candida albicans*.

Sama and Ajaiyeoba (2006) studied the antimicrobial activities of ethanol extracts of aerial part (stem and leaf) of *Capparis thonningi* and *Capparis tomentosa* and cleared that, the ethanol extract of *Capparis tomentosa* inhibited growth of *Staphylococcus aureus*, *Bacillus cereus*, *Aspergillus flavus* and *candida albicans*. The ethanol extract from *Capparis thonningi* made inhibition growth of *Staphylococcus aureus* and no sensitive activity against *Escherichia coli, Candida albicans* and *Aspergillus flavus.* Also, the antimicrobial activities of chloroform fraction from root of *Boscia angutifolia* (Capparaceae) studied by Hassan *et al*. (2007)who stated the same effects on *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Streptococcus pneumonia,*Chopade *et al.* (2008) reported that the roots of *Capparis zeylanica*were successively extracted with petroleum ether, chloroform, ethanol and water. All extracts had antimicrobial activities against *Bacillus pumilus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *proteus valgaris*, *Candida albicans* and *Aspergillus niger.*

Materials and Methods

Preliminary Phytochemical Screening:

Capparis sinaica. samples were collected from south Sinai Abu-Zanimah habitat and the experiments were done in Desert Research Center during the growth period 2006, cleaned, air-dried at lab-temperature and ground to fine powder, to be used for the following investigations: For testing the volatile oils, carbohydrates or glycoside, resin, chloride & sulphate, saponine, tannin, flavonids, sterol and terpenes, alkaloids, cardic glycoside according to Woo *et al.*, (1977); Markham (1982); Balbaa (1986); Hostettman *et al*., (1991); Silva *et al.,* (1998) andA.O.A.C., (2000).

Determination of total metabolic products

Determination total carbohydrates content were estimated according to Chaplin & Kennedy (1994), total nitrogen and protein (James, 1995) and total lipids content (Christie, 1982).

Determination of total active constituent

Total flavonoids, total tannins and saponins and total alkaloids content were determined according to Karawaya & Aboutable (1982), Honerlogen and Tretter, (1979) and Woo *et al*., (1977), respectively.

Investigation of flavonoids

The method of isolation of flavonoids depends on both plant source and type of flavonoid to be isolated. More polar solvents are used for extraction of the flavonoid glycosides (Smith *et al.,* 1966). The extract was fractionated using column and paper chromatography to obtain separately bands, each of them contain one compound, each compound finally purified and identified according to Anderson & Sowers, (1968); Stahl, (1969); Mabry *et al.*, 1970; Markham and Mabry (1975); Markham, (1982) and Harborne (1984).

Investigation of alkaloids

Melting points were determined on Fisher-johns and are uncorrected. UV spectra were taken on a Philips PYE Unican Pu 8800 Spectrophotometer. IR spectra were measured with a Nicolet 5700 of thermo. NMR spectra were measured in DEMSO-*d*6 on a VARIAN INOVA 600 spectrometer with chemical shifts being represented in parts per million (ppm) and tetramethylsilane (TMS) as an internal stander. EL-MS and HR-EL-MS were measured on a Zabspec E spectrometer at an ionization voltage of 70 ev. The TLC and HPTLC employed precoated silica gel plates (Qingdao Haiyang chem., co., Ltd.). For the column chromatography, silica gel (Qingdao Haiyang) and sephadex LH-20 (Pharmacia) were used X-ray crystallographic data by use MAC DIP-2030K single crystal X-ray diffract meter Harborne (1984).

Antimicrobial studies

The effect of different successive extracts of *Capparis sinaica* Veill. plant using the following solvents: hexane, benzene, chloroform, ethyl acetate, methyl alcohol, and water on some pollutant micro-organisms were achieved. Two bacterial strains (grame+ve) of *Bacillus subtilis, Staphylococcus aureus* and one bacterial strain (grame-ve)of *Escherichia coli* were tested. Beside seven fungal strains of *Aspergillus niger, Aspergillus flavous, Rhizopious sp., Fusarium oxysporium, pencilium sp., Candida albicana* and *Alternaria tennius* were also tested. The microorganisms were obtained from Botany and Microbiology Department, Faculty of Science, AL-Azher University, Cairo, Egypt. It was checked for purity, identity and regenerated to obtain active microorganisms. The cultures were stored in refrigerator at 5ºC and reactivated on the media suitable for each microorganism. Preparation of the Spore Suspension were described according to Padwal *et al.,* (1976). Agar diffusion method was used to check the presence or absence of antimicrobial agents as described by Booth (1972).

Results and Discussion

Preliminary Phytochemical Screening:

The preliminary phytochemical screening of *Capparis sinaica* showed that the plant contained: tannins, sterols, flavonoids, glycosides, saponines, chlorides, sulfate, resin, and alkaloids in all plant parts, but no volatile oil in all parts, (table 1).It is clear that, the flower and the aerial parts are rich in flavonoids, glycosides, cardiac glycosied and alkaloids, this is in agreement with Daniel 2005 and Sama &Ajaiyeoba 2006, who mentioned that, screening many species from family Capparaceae contains flavonoids and phenolic constituents.

Table 1: Preliminary phytochemical screening of *Capparis sinaica*.

Test	Aerial part	Flower	Root
Volatile oil	-ve	-ve	-ve
Carbohydrate or Glycoside	++ve	++ve	+ve
Resin	+++ve	+ve	+ve
Chloride	++ve	++ve	+ve
Sulfate	++ve	++ve	+ve
Saponin	+++ve	++ve	+ve
Tannins	++ve	+ve	+ve
Flavonoids	++ve	+++ve	+ve
Sterols	+ve	+ve	+ve
Alkaloids	+++ve	+++ve	+ve
Cardiac glycoside	+ve	++ve	-ve

-ve = negative result +ve = weak positive ++ve = moderate positive +++ve = strong positive

Determination of total metabolic products

Total carbohydrates content (%)

Data in Table 2 showed that the percentages of total carbohydrates of ***Capparis sinaica*** reached maximum values (1.94, 1.81 and 1.82 %) in aerial part, flower and root during winter, spring, and autumn, respectively, while reached to the least value 1.17 % in the roots during summer.

Total nitrogen and protein content (%)

Data presented in Table 2 indicated that *Capparis sinaica* flower contained the highest percentages of total nitrogen and total protein which recorded 2.3 % and 14.38 %, respectively. The decrease of total nitrogen and total protein during summer may be attributed to the decreased in the water content, which was found to be linked with an accumulation of some amino acids (e.g. proline), that may play an important role in increasing cell osmoregulation (Ali *et al*., 1992). The percentages of total protein for areal part showed the same trend for total nitrogen, where they reached to 8.44 and 8.18% in winter and spring, respectively. It was observed from the obtained results that the percentages of total nitrogen reached their maximum values, which may be attributed to the increase of water resources of the soil followed by increasing in metabolic rate (Stocker, 1960).

Total lipids content (%)

Capparis sinaica accumulated high amount of total lipids reached to its maximum values in autumn (9.50 and 5.80 %) in aerial part and root, respectively, while the flower contained (4.20 %) of lipid in spring as shown in Table 2.

Table 2: Mean values of total metabolic products of *Capparis sinaic* plant parts during the period of investigation (2006).

Item	Season	plant part		
		Aerial part	Flower	Root
Total carbohydrates	Winter	1.94	-	1.72
	Spring	1.75	1.81	1.46
	Summer	1.35	-	1.17
	Autumn	1.81	-	1.82
Total nitrogen	Winter	1.35	-	0.63
	Spring	1.31	2.3	0.56
	Summer	0.63	-	0.38
	Autumn	0.73	-	0.45
Total protein	Winter	8.44	-	3.93
	Spring	8.18	14.38	3.5
	Summer	3.93	-	2.73
	Autumn	4.56	-	2.81
Total lipids	Winter	5.14	-	3.8
	Spring	6.10	4.20	4.02
	Summer	7.82	-	4.18
	Autumn	9.50	-	5.8

Determination of total active constituent

Total flavonoids or glecosides content (%)

Total flavonoids present in different parts of *Capparis sinaica* Veill. (Aerial part, flower and root) at different growth seasons were determined spectrophotometrically and calculated as question. The percentages of total flavonoids reached their maximum values in winter for aerial parts and roots (0.82 and 0.33%), respectively. While the total flavonoids of flowers, reached to (0.34%) during spring (Table 3).

Total tannins content (%)

The tannins are waste-products of metabolism.When tannins are widely distributed within the plant, the amounts may vary greatly from one organ to another (Balbaa *et al*., 1981). The leather was generally cured with tannins derived from many plants, beside tannins were used for treatment of liver oliment by daily intake (Acheson *et al*., 1970). Hence we determined the content of tannins in *Capparis sinaica* to evaluate the economical values of the plant.

The percentages of total tannins (Table 3) showed that the total tannins reached their maximum values in plant aerial part during winter, this is may be due to high water resources (rainfall) leading to high metabolic rates, which agreed with (Ammar *et al*., 2004). The percentages of total tannins of of *Capparis sinaica,* flower (3.30%) was the highest value of tannins than that of the aerial parts and the root.

Total saponins content (%)

The percentage of total saponins (Table 3) present in *Capparis sinaica* plant during the year of investigation was reached to maximum values in the flower (1.37 %), and the minimum values were during summer in the plant root (0.31%).

Saponins are groups of steroids or triterpenes natural products occurring in many plant families. The ecological roles of this group have been defined with regard to certain plant microbes, plant-plant or plant-insect interactions. A function and insect predation suggests that they may have fungicidal and insecticidal roles in nature. Differential synthesis and /or accumulation of saponins and their aglycones are observed in different plant tissues and organs depending upon species or genotype, age and environmental conditions (Inderjit and Foy, 1999).

Total alkaloids content (%)

Data presented in Table 3 indicated that the percentage of total alkaloids present in *Capparis sinaica* plant during the year of investigation reached to the highest value (0.63 %) in flower than the parts during spring, while the maximum value of alkaloids in the aerial part and the roots were (0. 33 and 0. 26%) in winter, and the minimum values was observed in the Autumn root.

Table 3: Mean values of the different active constituent of *Capparis sinaica* plant parts during the period of investigation (2006).

Item	Season	plant part		
		Aerial part	Flower	Root
Total flavonoids	Winter	0.82	-	0.33
	Spring	0.75	0.34	0.28
	Summer	0.15	-	0.13
	Autumn	0.63	-	0.18
Total tannins	Winter	1.71	-	1.75
	Spring	1.68	3.30	1.67
	Summer	1.39	-	1.50
	Autumn	1.56	-	1.61
Total saponins	Winter	0.75	-	0.62
	Spring	0.53	1.37	0.52
	Summer	0.21	-	0.31
	Autumn	0.42	-	0.45
Total alkaloids	Winter	0. 33	-	0. 26
	Spring	0. 25	0.63	0. 20
	Summer	0. 11	-	0. 15
	Autumn	0.19	-	0.09

Investigation of flavonoids

The developed of methanolic extract of *Capparis sinaica* flowers was subjected to paper chromatography using BAW system (4:1:5) and AcOH 15 %, the dried chromatography, examined under UV light and re-examined after exposure to ammonia, revealed the presence of a complex pattern of phenolic constituents, most of them possessed flavonoid in nature.

The residue of the methanolic extract was washed with ethanol to get rid of salt (desalting process), and divided fraction into two main parts, the first main part was applied on the top of silica gel column chromatography, eluted firstly with chloroform, followed by ethyl acetate to increase polarity until pure ethyl acetate. It was followed by ethyl acetate/methanol until pure methanol, then it was followed by methanol/water until finally pure water, where two main fractions I and II. On the other hand the second main extract was obtained washed with successive selective organic solvents, benzene, chloroform, ethyl acetate, and finally ethanol, where two main fractions were obtained; benzene fraction and ethyl acetate fraction. Each fraction were purified to obtain the pure compounds (from A_1 toA_5). After we obtained the pure compound we identified them by using R_f values, UV spectral data, ^{1}H-NMR spectral data.

Compound A_1: compound A_1 has R_f values (0.71& 0.27) and color reaction are within the range of flavonoid aglycone (Table 4). UV spectral data of compound A_1 in methanol showed maximal absorption band I at 375nm, which indicated that compound A_1 is a flavonol compound with free 3-OH group. Formation of new band at 332nm.with NaOMe indicated the presence of a free 7-OH group. A bathochromic shift of band I when treated with $AlCl_3$ indicated the presence of 3 and 5-OH group. A bathochromic shift with HCl in band I indicated the presence of O-dihydroxy group in B-ring.Bathocromic shift in band I with $NaOAc/H_3BO_3$ was an additional proof for the presence of O-dihydroxyl group in B-ring (Table 5).

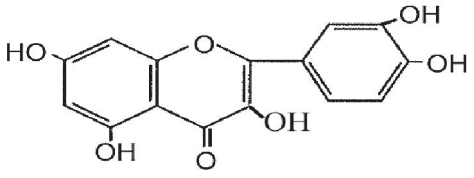
The structure of compound A_1 was further confirmed as quercetin by ^{1}H-NMR spectrum (Table 6), which showed characteristic signals for quercetin. Thus, compound A_1 was identified as quercetin**.**

^{1}H-NMR spectral data (Table 6): The ^{1}H-NMR spectrum of compound A_1 in $CDCl_3$ (Fig. 15) showed signals atδ(ppm) δ 7**.**7 (1H, d, J=8**.**1 Hz, H-2\), δ 7**.**6 (1H, dd, j = 8**.**5 Hz, H-6\), δ 6**.**89 (1H, d, j = 8**.**5 Hz, H-5\), δ 6**.**4 (1H, d, j = 2**.**5 Hz, H-8) and δ 6**.**2 (1H, d, j = 2**.**5 Hz, H-6)**.**

The structure of compound A_1 was further confirmed as quercetin by ^{1}H-NMR spectrum, which showed characteristic signals for quercetin. Thus, compound A_1 was identified as quercetin**.**

The change of its color from yellow to light yellow (Table 4) when exposed to ammonia vapor under UV light or treated with $AlCl_3$ reagent indicated that A_1 might be a flavonol compound with free OH (Harborne, 1984 and Liu *et al.,* 1989).

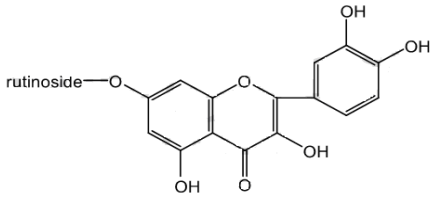
Quercetin



Compound A_2: Compound A_2 was obtained, its R_f values were (0.21& 0.75) and color reaction are within the range of flavonoids (Table 4).UV spectral data (Table 5) of compound A_2 in MeOH showed maxiumum absorption band I at 357 nm which indicated that compound A_2 is a flavonol compound. A_2 bathochromic shift in band I (+67) formation a new band at 424 nm when add NaOMe indicated that no free OH in 4' position. When add NaOAc no shift in band II so that there is no free oH in 7 or 7 occubied. By add H_3BO_3 A bathochromic shift in band I (+24) indicated that free OH group in 3', 4'. A band I with $AlCl_3$ make bathochromic shift (+24) and this indicated free OH in 3, 5. with HCL Ahypthochromic shift in band I (+79) this due to free OH in 3', 4' position.

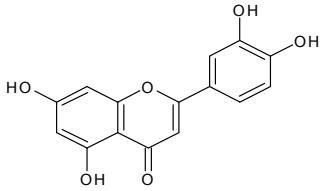
The structure of compound A_2 was further confirmed as Quercetin-7-o-rutinoside, by ^{1}H-NMR spectrum (Table 6) which show a characterectic signal of quercetin-7-O-rutinoside.^{1}H-NMR spectrum (Table 6)showed quercetin skeleton, as in the B-ring, the H-5' proton shifted upfield (at δ 6.8 ppm) from the H-2′, H-6' protons as a doublet and the H-2', H-6' protons appear downfield from the H-5′ proton (at δ 7.32, 7.35 ppm respectively), H-6 and H-8 in A-ring show downfield (at δ 6.38, 6.59 ppm respectively) as a result of substitution at position 7. Sugar moiteties: δ (ppm) 5.05 (d, j=7.5Hz for H-1glucose), 4.55 (d, j=2.5 Hz for H-1‴ rhamnose at 7-position) 3.1-3.9 (m) for the rest of two sugar protons and 0.95 (d, j=6 Hz for rhamnosyl CH_3). Acid hydrolysis of the compound A_2 by 0.1 N HCl (controlled or mild acid hydrolysis) afforded quercetinas the aglycone. The sugar moiety was identified as rutinoside with authentic pure sugars.From the above mentioned data and by comparing with the published data (Mabry *et al.*, 1970), compound A_2 was confirmed to be identified asquercetin-7-O-rutinoside.

Quercetin-7-O-rutinoside



Compound A_3: Compound A_3 was obtained, its R_f values (0.75& 0.10) and color reaction (Table 4) are within the range of flavonoid. UV spectral analysis of compound A_3 (Table 5): Band I appeared at 349 nm and band II at 253 nm indicated that the compound may be flavones in nature. Bathochromic shift of band I with NaOMe (+52 nm) with an increase of intensity indicated the presence of free OH group at position 4'. Bathochromic shift in band II with addition of NaOAc (+16 nm) pointed out the presence of free OH group at position 7. Bathochromic shift in band I with addition of NaOAc/ H_3BO_3 (+21 nm) indicated the presence of orthodihydroxy groups at B-ring. Bathochromic shift in band I with addition of $AlCl_3$ (+77 nm) indicated the presence of free OH group at position 5 and/or orthodihydroxy group at B-ring. Hypsochromic shift in band I in $AlCl_3$ spectrum after the addition of HCl indicated the presence of orthodihydroxy groups.^{1}H-NMR spectrum (Table 6)of compound (A_3) in DMSOd6 showed the characteristic signals of luteolin nucleus at: δ (ppm) 7.9 (1H, dd, J= 2.8; 7.5Hz, H-6` ), δ 7.84 (1H, d, J= 2.5Hz, H-2`), δ 7.48 (1H, d, J= 8.4Hz, H-5`), δ 6.7 (1H, d, J= 2.5Hz, H-8), δ 6.6 (1H, s, H-3) and δ 6.15 (1H, d, J= 2.5Hz, H-6).
From R_f values, colour reaction, UV spectrum and ^{1}H-NMR; compound A_3 is luteolin free (Mabry *et al.*,1970).

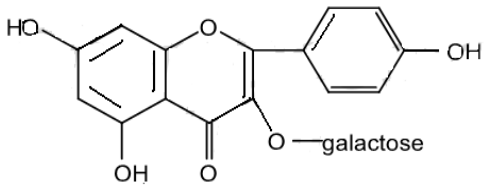
Luteolin



Compound A_4: Compound A_4 was obtained, its R_f values (0.76& 0.11) and color reaction (Table 4) are within the range of flavonoid. U.V. spectral data (Table 5) of compouned A_4 in methanol showed maxiumum absorption band I at 348nm which indicated that A_4 is flavonol with 3-occupeied. A bathochromic shift in band I (+54) formed a new band at 402 nm when add NaOMe. A bathochromic shift in band I so that there is a free OH in 4' position. NaOAc made abathochromic shift in band II (+8) and this indicated for the presence of free OH in 7. no shift in band I occur by adding H_3BO_3 so that no free OH group in 3', 4' position. A bathochromic shift in band I (+52) by add $AlCL_3$ and this indicated the free OH in 3,5. HCL adding make no shift in band I and this indicated no free OH 3', 4'.This compound A_4 identified as kaempferol-3-galactose.

The ^{1}H-NMR spectrum (Table 6)of the compound F_5 in DMSO (dimethyl sulfoxide) showed that, signals at δ ppm 3.1-3.7 (m, protons of sugar), 5.4(1H, d, J= 8Hz, protons of glucose H-1$^{\backslash\backslash}$), 6.23 (1H, d, J= 2.1Hz, H-6), 6.45 (1H, d, J= 2.4Hz, H-8), 6.9 (2H, d, J= 2Hz, H-3$^{\backslash}$, H-5$^{\backslash}$) and 8.05 (2H, d, J= 8Hz, H-2$^{\backslash}$, H-6$^{\backslash}$).

Kaempferol-3-galactose



Compound A_5: Compound A_5 was obtained, its R_f values (0.75& 0.9) and color reaction (Table 4) are within the range of flavonoid.UV spectral data (Table 5)of compound A_5in MeOH (258, 268(sh), 358 nm) indicated a possible flavonol skeleton (flavonol with free hydroxyl group at position 3). On addition of NaOMe it exhibited a bathochromic shift in band I (+51 nm), indicating a free OH group at position 4'. The lack of shift in band II on the addition of NaOAc indicating that C-7 position is occupied. On addition of $AlCl_3$ it exhibited a bathochromic shift in band I (+57 nm) which disappeared after the addition of HCl, indicating the presence of a free OH group at C-5 and orthodihydroxy pattern at B-ring (C-3', C-4'), which was further confirmed by sodium acetate/ boric acid spectrum, a bathochromic shift in band I (+12 nm) occurred.^{1}H-NMR spectrum (Table 6)showed quercetin skeleton, as in the B-ring, the H-5' proton shifted upfield (at δ 6.8 ppm) from the H-2', H-6' protons as a doublet and the H-2', H-6' protons appear downfield from the H-5' proton (at δ 7.32, 7.35 ppm respectively), H-6 and H-8 in A-ring show downfield (at δ 6.38, 6.59 ppm respectively) as a result of substitution at position 7. Signals also indicate the presence of glucose.

Acid hydrolysis of the compound A_5 by 0.1 N HCl (controlled or mild acid hydrolysis) afforded quercetin as the aglycone. The sugar moiety was identified as glucose with authentic pure sugars.

From the above-mentioned data and by comparing with the published data (Mabry *et al.*, 1970), compound A_5 was confirmed to be identified asquercetin-7-*O*-glucoside.

Quercetin-7-*O*-glucoside

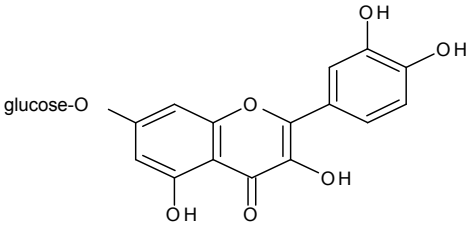


Table 4: R_f value and colourreactionof the isolated compounds.

		A_1	A_2	A_3	A_4	A_5
R_f value in solvent systems	BAW	0.71	0.21	0.75	0.76	0.75
	AcOH-15%	0.27	0.75	0.10	0.11	0.9
Colour of spot	UV	yellow	yellow	deep Purple	yellow	yellow
	UV + NH_3	brown	brown	yellow	deep brown	deep brown
	$AlCl_3$	yellow	yellow	yellow	deep yellow	deep yellow

Table 5: UV spectral data λ_{max} nm of the isolated compounds.

	UV data					
	MeOH	MeONa	$AlCl_3$	$AlCl_3$/ HCl	AcONa	AcONa/ H_3BO_3
A_1	260, 270 (sh.),300 (sh.), 375	290,370(sh.), 450	276, 318, 450	273,305(sh.),355(sh.), 427	265, 332(sh.), 383	260,300(sh.), 380.
A_2	261, 357	275, 424	274, 436	274, 400	266, 264, 381	299, 264, 381
A_3	253, 349	266, 329, 401	274, 328, 426	275, 355, 358	269, 326, 384	259, 370
A_4	267, 324, 348, 440	275, 324, 402	274, 305,370, 400	274, 303, 356, 400	275, 305, 384	268, 352
A_5	258, 268(sh), 358	273, 409	270, 323(sh), 415	272, 352, 374	258(sh), 267, 372	259, 370

Table 6: ^{1}H-NMR spectrum δ (ppm) in DMSO-$_{d6}$ (400 MHz) data of the isolated compounds.

	^{1}H-NMR spectrum data
A_1	δ 7.7(1H, d, J=8.5Hz, H-2\), 7.6 (1H, d, J=8.5Hz, H-6\), 6.89 (1H, d, J=8.5Hz, H-5\), which indicated the presence of aromatic ring with two substitution at m,p- substitution, δ 6.4 (1H, d, J=2.5Hz, H-8) and 6.2 (1H, d, J=2.5Hz, H-6). (m).
A_2	Δ 7.35 (1H, d ,j=8.5 Hz, H2, H-6'), 7.32 (1H, d, j=8.5, H2, H-2'), 6.8 (1H, d, j= 8.5Hz, H-5), 6.59(1H, d, j=2.5Hz, H-8), 6.38(1H, d, j=2.5 Hz, H-6). Sugar: 5.05 (d, j=7.5Hz for H-1glucose), 4.55 (d, j=2.5 Hz for H-1‴ rhamnose at 7-position) 3.1-3.9 (m and 0.95 (d, j=6 Hz for rhamnosyl CH_3).
A_3	Aglycone:δ 7.9 (1H, dd, J= 2.8; 7.5Hz, H-6'), 7.84 (1H, d, J= 2.5Hz, H-2'), 7.48 (1H, d, J= 8.4Hz, H-5'), 6.7 (1H, d, J= 2.5Hz, H-8), 6.6 (1H, s, H-3), 6.15 (1H, d, J= 2.5Hz, H-6).
A_4	δ 8.05(2H, d, j= 8Hz, h-2', h-6'), 6.9(2H, d, j=2 Hz, H-3', H-5'), 6.45(1H, d, j=2.4 Hz, h-2), 6.23(1H, d, j=2.1 Hz, H-6). Sugar:δ 5.4 (1H, d, j= 8Hz, H-1" glucose), remaning sugar protons are evident at 3.1-3.7ppm(m).
A_5	Aglycone:δ 7.35 (1H, dd, J= 8.5; 2.5Hz, H-6'), 7.32 (1H, d, J= 2.0Hz, H-2'), 6.8 (1H, d, J= 8Hz, H-5'), 6.59 (1H, d, J= 1.5Hz, H-8), 6.38 (1H, d, J= 1.5Hz,H-6).Sugar: δ 5.1 (1H, d, J= 10Hz, H-1" glucose), remaining sugar protons are evidant at 3.04-4.01ppm (m).

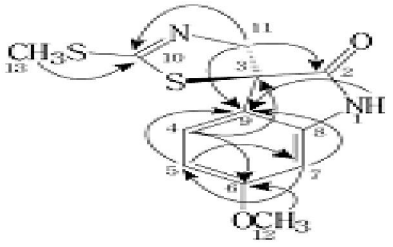
Investigation of alkaloids

The air-dried plant material (1 kg) was powdered and extracted with 95% EtOH and 50% EtOH respectively. The alcohol extract were concentrated under reduced pressure and successively extracted with petroleum ether, $CHCl_3$, EtOAc and n-BuOH. The $CHCl_3$-soluble extract (60.8 g) was subjected to a silica gel column chromatography and eluted with petroleum ether/EtOAc (gradient, 1 : 0→0 : 1) to yield 26 fractions. (3.7 g) were further separated with silica gel column using petroleum ether/EtOAc (gradient, 1: 0→0: 1) to yield 43 portions, and portion27 was purified by Sephadex LH-20 to afford 4 (18 mg). (1.5 g) were further separated with silica gel column using petroleum ether/EtOAc (gradient, 1: 0→0: 1) and Sephadex LH-20 to yield 2 (12 mg) and 5 (5 mg). (5 g) were applied to silica gel column eluted with a gradient of petroleum ether/EtOAC and Sephadex LH-20 to give 1 (13 mg) and 3 (9 mg). EtOAc-soluble extract (116.3 g) was subjected to a silica gel column eluted with $CHCl_3$/MeOH (gradient, 1 : 0→0 : 1) to yield 28 fractions. (4 g) was further separated with silica gel using petroleum ether/acetone (gradient, 1: 0→0: 1) and Sephadex LH-20 to yield 6 (14 mg) and 7 (23 mg), were separated with Sephadex LH-20 to yield 8 (25 mg) and 9 (10 mg), respectively.

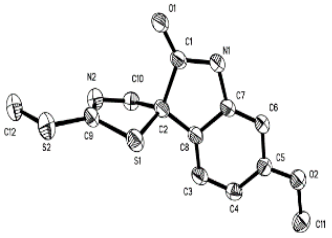
Compound 1: Pale yellow plates crystal ($CHCl_3$–CH_3OH), mp 170—173 °C; [a]D 25 9.88° (c_0.16, MeOH); UV l max (MeOH) nm (loge): 224 (3.34), 198 (2.86); HR-EI-MS m/z: 280.0341 [M_] (calculated for $C_{12}H_{12}N_2O_2S_2$, 280.0340); EI-MS m/z (%): 280 (M_, 73), 233 (22), 207 (100),175 (51), 162 (16); IR (cm_1): 3436, 3169, 3057, 2926, 2835, 1715,1587, 1505, 1463, 1344, 1281, 1159; ^{1}H-NMR (DMSO-d6, 600 MHz) and ^{13}C-NMR (DMSO-d6, 150 MHz) see (Table 7).Crystals of compound 1 was crystallized from $CHCl_3$–CH_3OH (1: 1). Crystal data: $C_{12}H_{12}N_2O_2S_2$, MW280.38, triclinic, space group P-1, a5.750 (1) Å, b 10.189 (2) Å, c11.988 (2) Å, a 106.96°, b_96.97°, g_102.59°, V 642.6 (3)Å3, Z 2, Dcalc1.449 g/cm^3, T283 K, Crystal size: 0.05 0.30 0.60 mm. Singlecrystal analysis of 1 was made on a MAC DIP-2030K diffractometer with monochromatedMoKa radiation (50 kV, 60 mA). A total of 1647 unique reflections were recoded, of which 1545 were considered observed on the basis$|F|^2$_2s $|F|^2$. The structure was solved by direct methods with the use of the SHELX-97 program, and all hydrogen atoms were located from a difference Fourier map. Final R-factors were R_0.0700 and Rw_0.1756.

Compound 1: was obtained as yellow plates, and gave a positive reaction to dragendorff reagent. The molecular formula of 1 was determined to be $C_{12}H_{12}N_2O_2S_2$ by HR-EI-MS(m/z 280.0341 [M]_). The IR spectrum displayed absorption bands for amide (3436 cm_1), carbonyl (1715 cm_1) and aromatic group (1615, 1505, 1463 cm_1), respectively. The ^{1}HNMR spectrum of 1 indicated the presence of a 1,2,4-trisubstituted benzene [d H 7.24 (d, J_8.4 Hz), 6.57 (dd, J_8.4, 1.8 Hz), 6.40 (d, J_1.8 Hz)] and a AB coupling system [d H 4.46, 4.36 (d, J_15 Hz)] corresponding to methylene (_N–CH_2–). In addition, the signal at d H 10.64 (s) showed the presence of an amidocyanogen. ^{1}H-NMR(Table 7) signals at d H 3.74 (s) and 2.50 (s) indicated the existence of a methoxyl and a methylthio, respectively. The ^{13}C-NMR spectrum exhibited 12 carbon signals which revealed the existenc of one carbonyl carbon atom (d C 177.5), one olefinicquaternary carbon (_C_N–) (d C 161.8), and one methylene (d C 73.6), one quaternary (d C 64.2), one methoxyl (d C 55.3),one methylthio (d C 15.0), and one benzene ring (d C 160.6, 142.4, 125.1, 121.4, 107.6, 96.7). ^{1}H-, ^{13}C-NMR and EI-MS data strongly suggested the feature of spirobrassinin derivative.17) According to the molecular formula and the structure of 1,2,4-trisubstituted benzene indicated in ^{1}H-NMR spectrum, a methoxyl was suggested located at C-5 or C-6. In the heteronuclear multiple bond connectivity (HMBC) spectrum (Fig. 10), the long-range coupling between H-12 (d H 3.74) and C-6 (d C 160.6); H-4 (d H 7.24) and C-3 (d C 64.2), C-6 (d C 160.6); H-5 (d H 6.57) and C-7 (d C 96.7), C-9 (d C 121.4); H-7 (d H 6.40) and C-5 (d C 107.6), C-9 (d C 121.4) indicated that the methoxyl was located at C-6. Thus1 was identified as 6-meth (methylthio) spiro [3H-indole-3,5_(4_H) thiazol]-2-one and all the ^{1}H and ^{13}C signals had been designed by 2D NMR. The relative structure of 1 was further confirmed by

single crystal X-ray diffraction studies (Fig. 10). We named this compound Capparine A. In addition, single crystal X-ray analyses of 1 showed that it was racemic while the specific rotation suggested that levorotary enantiomer was a little bit more than dextrorotary enantiomer. One possible explanationfor contradictory fact is that the sample used for specific rotary determination is obtained before single crystal formation, which contains more levorotatory enantiomer than dectrorotatory enantiomer. However, the single crystal grew for single crystal X-ray diffraction study was a result of the combination of levorotary enantiomer and dextrorotary in a 1: 1 ratio. Since 1 was too littleto resolute the anantiomers, we will proceed with the study in our continuous work.



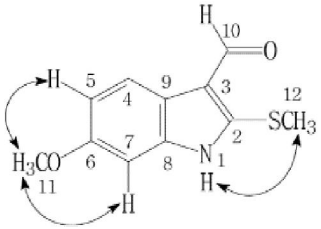
Key HMBC correlation of compound 1



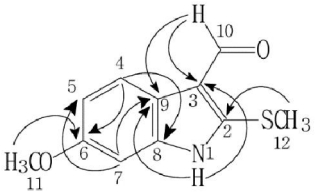
The X_Ray Crystallographic Structure of compound 1

Compound 2: White amorphous powder ($CHCl_3$–MeOH), mp 202— 204 °C (dec); UV l max (MeOH) nm (loge): 333 (2.83), 284 (2.97), 247 (3.17), 223 (3.17), 192 (3.29); HR-EI-MS m/z: 221.0518 [M_] (Calcd for $C_{11}H_{11}NO_2S$, 221.0511); EI-MS m/z (%): 221 (M_, 100), 206 (68), 178 (18); IR (cm_1): 3092, 2949, 1618, 1581, 1515, 1453, 1349, 1146, 1092, 828, 726; ^{1}H-NMR (DMSO-d6, 600 MHz) and ^{13}C-NMR (DMSO-d6, 150 MHz).

Compound 2: was obtained as white amorphous powder.The molecular formula (Table 7) was determined to be $C_{11}H_{11}NO_2S$ by HR-EI-MS (m/z 221.0518 [M]_). The IR spectrum displayed absorption bands for carbonyl (1618 cm_1) and aromatic group (1581, 1515, 1453 cm_1). The 1H-NMR spectrum indicated the presence of 1,2,4-trisubstituted benzene [d H 7.87 (d, J_9.0 Hz), 6.83 (dd, J_9.0, 2.4 Hz), 6.90 (d, J_2.4 Hz)], a methoxyl (d H 3.80, s), a methylthio (d H 2.66, s), an amidocyanogen (d H 12.11, br s) and an aldehyde group (d H 10.01, s).



Key HMBC Correlation of compound 2



Key Noesy correlation of compound 2

^{13}C-NMR spectrum exhibited 11 carbon signals. In the HMBC spectrum (Fig. 31), the correlation between H-1 (d H 12.11) and C-3 (d C 115.9), C-9 (d C 119.4); H-4 (d H 7.87) and C-6 (d C 156.5), C-8 (d C 137.8); H- 7 (d H 6.90) and C-9 (d C 119.4), C-5 (d C 111.5); H-11 (d H 3.80) and C-6 (d C 156.5) indicated the existence of 6-methyloxy- indole, the long-range coupling between H-10 (d H 10.01) and C-9 (d C 119.4), C-3 (d C 115.9) suggested an aldehyde group connected with C-3, the correlation between H- 12 (d H 2.66) and C-2 (d C 143.9) indicated a methylthio connected with C-2. Furthermore, the NEOSY spectrum confirmed the methylthio and the methoxy connected with C-2 and C-6, respectively by correlations of H-1/H-12, H-11/H-5 and H-11/H-7.

Thus compound 2 was identified as 6- methoxy-2-(methylthio)-1H-indole-3-carbaldehyde and all the ^{1}H and ^{13}C signals had been designed by 2D NMR. We named this compound Capparine B.

Table 7: ^{1}H- and ^{13}C-NMRSpectral data of Compounds 1 and 2.

Position	1		2	
	δH (*J* in Hz)	δ C	δH (*J* in Hz)	δ C
1	10.46, s		12.11, s	
2		177.5		143.6
3		64.2		115.9
4	7.24, d (8.4)	125.1	7.87, d (9.0)	120.3
5	6.57, dd (8.4, 1.8)	107.6	6.83, dd(9.0, 2.4)	111.5
6		160.6		156.5
7	6.40, d (1.8)	96.7	6.90, d (2.4)	94.9
8		142.2		137.8
9		121.4		119.4
10		161.8	10.01, s	183.1
11	A4.46,d(15.0):4.36,d(15.0)	73.6	3.80, s	55.2
12	3.74, s	55.3	2.66, s	16.7
13	2.50, s	15.0		

Antimicrobial studies

Successive extraction using different organic solvent (hexane, benzene, chloroform, ethyl acetate, methanol, and water) carried out on total alcoholic extract of different parts of *Capparis sinaica*. Those extracts were tested against different strains of bacteria and fungi, the activity of extracts against bacteria and fungi were determined through diameter of inhibition zones.

Anti-bacterial activity

It was observed from the obtained results at Table 8 that the ethyl acetate and methanol flower extracts and ethyl acetate root and methanol aerial part extracts of *Capparis sinaica* showed the best inhibition effect against *Bacillus subtilis, Staphylococcs aureus*, *Escherchia coli*, followed by chloroform.

On the other hand, it was found that benzene fraction of the all parts under investigation (Aerial part, flower and root) of *Capparis sinaica* gave negative effects against *Bacillus subtilis, Staphylococcs aureus*, *Escherchia coli.*

Table 8: The effect of plant part extracts on different fungal and bacterial strains (diameter of inhibition zone by cm).

		E. coli.	*Staphylococcus aureus*	*Bacillus subtilis*	*Candida albicana*	*Aspergillusniger*	*Aspergillusflavous*	*Alternariatenuis*	*Rhizopious sp.*	*Fusariumexosporum*	*Peniccilum sp.*
Aerial part	Hexan	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	Benzene	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	Chloroform	1.1	1.3	1.1	0.6	-ve	-ve	-ve	-ve	-ve	-ve
	Ethyl acetate	-ve	-ve	-ve	-ve	1.9	0.5	0.6	0.6	0.7	0.5
	Methanol	1.9	1.9	2.0	2.1	0.5	0.4	0.5	0.6	0.5	0.5
	Water	1.3	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Flower	Hexane	1.1	1.2	1.1	1.0	-ve	-ve	-ve	-ve	-ve	-ve
	Benzene	-ve	-ve	-ve	-ve	-ve	-ve	-vce	-ve	-ve	-ve
	Chloroform	1.2	1.2	1.3	0.6	-ve	-ve	-ve	-ve	-ve	-ve
	Ethyl acetate	1.9	1.8	1.9	1.9	0.6	0.5	-ve	0.5	0.6	0.7
	Methanol	1.8	1.9	1.9	2.00	-ve	-ve	0.6	0.5	-ve	-ve
	Water	-ve	-ve	-ve	-ve	0.8	-ve	-ve	-ve	-ve	-ve
Root	Hexane	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	Benzene	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	Chloroform	0.6	1.3	0.6	0.5	-ve	-ve	-ve	-ve	-ve	-ve
	Ethyl acetate	1.8	1.7	2.5	1.9	0.5	0.5	0.6	0.7	0.6	0.6
	Methanol	1.2	1.2	1.3	1.1	-ve	-ve	-ve	-ve	0.5	-ve
	water	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Anti-fungal activity

As shown in Table 8, extracts of ethyl acetate, methanol of aerial part of *Capparis sinaica* plant produced the best inhibition effects against the all tested fungi spices, while each of hexane, benzene, chloroform and water of all fractions have not any anti-fungi activities against different fungi strains.

On the other hand, it is known that *Bacillus subtilis* cause food poising, so the plant extract may be used in protecting from food poisning, *Staphylococcus aureus* and *Echerichia coli* cause skin infection and intestine tract infection, stoke plant extract may be used in the treatment of skin infection and intestine tract infection. All fungi tested were causes skin infection in case of immune deficit patient there were causes fungal disease e.g.: pulmonary infection in human. Meanwhile the *Aspergillus niger* was causes rotten of citrus fruits, while *Fusarium sp.* was cause rotten of roots and weltering of plant. *Candida albicana* causes vaginits, urinary tract infection, so the plant extract may be used in the treatment of skin infection and may be used as anti-fungal.

As a general conclusion, it could be concluded from the previous results that, *Capparis sinaica* Veill. Plant extracts have high antimicrobial activity, which may be can used as a natural safe source for manufacture saving materials or drugs for curing and protecting the human, the animals and the plants from the diseases and pollution attacking them.

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