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Full Length Research Paper

Pharmaceutical prospecting of natural products from the genus *Amberboa*

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Pharmaceuticals have attracted increased attention because of their beneficial effects on human health. A large number of secondary metabolites with various biological activities have been discovered but the potential for industrial development as drugs and nutritional supplements are limited. However, some bioactive substances derived from plants have diverse functional roles as secondary metabolites and these properties can be applied to the developments of novel pharmaceuticals. Recently, extensive studies have been conducted on the general aspects of the chemical structures, physical and biochemical properties and biotechnological applications of bioactive substances derived from various plants. In this research, we have summarized the pharmaceutical prospecting of natural compounds such as cycloartanes, flavonoids, sesquiterpenes, fatty acids, lignans, sterols and other metabolites isolated from the genus *Amberboa* and their progresses in biotechnological applications as pharmaceuticals.

Key words: Cycloartane, flavonoids, sesquiterpenes, fatty acids, lignans, sterols, other metabolites, pharmaceuticals, *Amberboa*.

INTRODUCTION

Throughout known history, the mankind has remained interested in natural medicines and plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years. These plant-based systems continue to play an essential role in health care, and it has been estimated by the world health organization that approximately 80% of the world's inhabitants, largely in the developing countries, rely mainly on traditional medicines for their primary health care. If one considers the therapeutic importance of morphine, quinine, digitalis, atropine, reserpine, vincristine, vinblastine etc., it is evident on how great is the contribution of plant derived drugs to medicine even in present era of science and technology (Newman et al., 2000; Cordell, 2000). Plants are excellent reservoir for identifying and extracting different secondary metabolites due to biodiversity, archaism of characters, organizational and behavioral patterns and the phytochemical studies on medicinal plants have served the dual purpose of bringing up new therapeutic agents, and providing useful leads for chemotherapeutic studies directed towards the synthesis of drugs modeled on the chemical structure of the natural products. Moreover, they prompted studies in the correlation of chemical structure and physiological activity through functional group variation in the constituents of the plant materials (Cordell, 2000).

Natural product research in the field of pharmaceutical development has witnessed a surprising expansion, and numerous biologically active compounds currently in clinical application are either natural products or derivatives thereof (Faulkner, 2002; Pietra, 1997; Laird and Altena, 2006). These naturally occurring secondary metabolites not only serve as drugs or leads for pharmaceutical development, but in many instances lead to the discovery of novel chemical synthetic methodologies and biological interaction pathways. Naturally occurring secondary metabolites are widely distributed in

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nature and have diverse significant biological activities (Hussain et al., 2009; Mehmood et al., 2008). *Amberboa* is a small genus of family Compositae, comprising only six species. Compositae is a vast family of flowering plants, distributed in all parts of the world. It comprises 800 genera and nearly 20,000 species. These are mostly annuals or perinnual herbs, a few are woody but not usually true trees. Many of its genera are prized among ornamentals (Krishnamurthi, 1969; Nasir and Ali, 1972; Dymock et al., 1891; Chopra et al., 1976; Nadkarni and Nadkarni, 1976; Kirtikar and Basu, 1918; Baquar, 1989; Hooker, 1982; Jafri, 1966).

The aim of this study is to review various aspects of natural products and their activities isolated from the genus *Amberboa* including cycloartanes, flavonoids, sesquiterpenes, fatty acids, lignans, sterols and other metabolites to explore the phytochemistry and pharmacological activities of genus *Amberboa* in order to collate existing information on this genus as well as highlight its multi-activity properties as a medicinal agent. Altogether, 58 compounds and 55 references are cited in this review article. All these compounds were isolated by column chromatography techniques (see materials and methods for explanation) and were elucidated by spectroscopic analysis (see results and discussion for explanation).

MATERIALS AND METHODS

General

The commercially available solvents were distillated and used for thin layer and column chromatographic techniques. Hoarse-serum butyrylcholinesterse (E.C 3.1.1.8), butyrylthiocholine chloride, 5, 5´-dithiobis [2-nitrobenzoic acid] (DTNB) and galanthamine were purchased from Sigma (St. Louis, MO, USA). Buffer and other chemicals were of analytical grade. All melting points were recorded in glass capillary tubes using a Büchi 535 melting point apparatus. Optical rotations were measured on a JASCO DIP-360 (Japan Spectroscopic Co. Ltd., Tokyo, Japan) digital polarimeter. The UV spectra were recorded on a Shimadzu UV-240 (Shimadzu Corporation, Tokyo, Japan) spectrophotometer. The IR spectra were recorded on a Shimadzu IR-460 (Shimadzu Corporation, Tokyo, Japan) instrument.

The 1H-NMR spectra were scanned on a Bruker AM 300 FT NMR, AM 400 FT NMR and AM 500 FT NMR spectrometers using TMS as an internal standard. The 13C-NMR spectra were recorded at 75, 100 and 125 MHz on a Bruker AM 300 FT NMR, AM 400 FT NMR and AM 500 FT NMR spectrometers, respectively. The mass spectra were scanned on a Varian-MAT 112s and Finnigan MAT-112 and 312A double focusing mass spectrometers connected to DEC PDP 11/34 and IBM-AT compatible PC based system, respectively. Electron impact, peak matching, field desorption (FD) and fast atomic bombardment (FAB) experiments were either performed on a Varian/Finnigan-MAT-312A or on a Jeol-JMS HX-110 mass spectrometers. FABMS were recorded in a glycerol-water (1:1) matrix in the presence of KI. High Resolution electron impact mass spectra (HREIMS) were recorded on a Jeol-JMS H X-110 mass spectrometer. Column chromatography was carried out on silica gel (type 60, 70 –230 mesh, E. Merck). Ceric sulphate reagent was used for the detection of compounds and aniline phthalate reagent for sugar's detection. Ceric sulphate (0.1 g) and trichloroacetic acid (1 g) were dissolved in 4 ml distilled water. The solution was boiled and conc. H_2SO_4 was added drop-wise until the disappearance of turbidity.

Extraction and isolation

The whole plant of *Amberboa ramosa* (20 kg) was chopped into small pieces, shade dried and ground. The powdered residue (12 kg) was extracted thrice with methanol. The combined methanolic extract was evaporated under reduced pressure and the resulting residue (900 g) was suspended in water and was repeatedly, extracted with hexane, chloroform, ethyl acetate and butanol. The chloroform (70 g) fraction was subjected to the column chromatography using hexane, hexane: ethyl acetate, ethyl acetate: methanol in increasing order of polarity to obtained sub-fractions A-F. The sub-fraction A was obtained through elution with hexane: ethyl acetate (8.3:1.7) and was subjected to flash chromatography eluting with hexane: ethyl acetate in increasing order of polarity. They eluent from hexane:ethyl acetate (8.8:1.2) afforded compound 3 and 46, respectively. The unresolved remaining fractions were rechromatographed over flash silica. Elution with hexane:ethyl acetate (8.5:1.5) provided 24. The sub-fraction B was obtained with hexane:ethyl acetate (7.3:2.7) and further subjected to flash column chromatography using hexane:ethyl acetate (8:2) as eluent which resulted in the isolation of 1, 2 and 5, respectively. The repeated chromatography resolution of unresolved fractions using hexane:ethyl acetate (7.8:2.2) led to the isolation of 4, 6 and 25 sucessively. The sub-fraction C was obtained through hexane:ethyl acetate (7.2:2.8) was subjected to the flash column chromatography using hexane:ethyl acetate (7.5:2.5) as eluent afforded 8 and 38, respectively. The Sub-fraction D was eluted with hexane:ethyl acetate (6.7:3.3) and further column chromatographic resolution over flash silica use hexane:ethylacetate (7.1:2.9) are afforded 7 and 37, respectively. The unresolved remaining fractions were rechromatographed over flash silica. Elution with hexane: ethyl acetate (6.9:3.1) was provided 27 and 39, respectively. The Sub-fraction E was eluted with hexane: ethyl acetate (5.5:4.5) and further column chromatographic resolution over flash silica using hexane: ethyl acetate (6.3:3.7) afforded 21 and 29, respectively. The unresolved remaining fractions were rechromatographed over flash silica. Elution with hexane: ethyl acetate (6:4) provided 22 and 23, respectively. The sub-fraction F was eluted with $CHCl_3$:MeOH (8.5:1.5) and further column chromatographic resolution over flash silica using $CHCl_3$:MeOH (9.2:0.8) afforded compound 47. The unresolved remaining fractions were rechromatographed over flash silica. Elution with $CHCl_3$:MeOH (8.7:1.3) was provided 36.

In vitro butyrylcholinesterase inhibition assays

Butyrylcholinesterase inhibition activity was measured by slightly modified spectrophotometric method developed by Ellman et al. (Khan et al., 2004a). In this assay protocol 150 µL of (100 mM) sodium phosphate buffer (pH 8.0), 10 µL of DTNB, 10 µL of test-compound solution and 20 µL of butyrylcholinesterase solution were mixed and incubated for 15 min (25°C). The reaction was then initiated by the addition of 10 µL butyrylthiocholine (substrate). The hydrolysis of butyrylthiocholine was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of butyrylthiocholine at a wavelength of 412 nm (15 min). Test compounds and the control were dissolved in MeOH. All the reactions were performed in triplicate in 96-well micro-plate in SpectraMax 340 (Molecular Devices, USA). Estimation of IC_{50} values: The concentrations of test compounds that inhibited the hydrolysis of substrate (butyrylthiocholine) by 50% (IC_{50}) were determined by monitoring the effect of increasing concentrations of these compounds in the assays on the inhibition values. The IC_{50}

values were then calculated using with the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA).

Assay of urease inhibition

Reaction mixtures comprising 25 μl of enzyme (Jack bean Urease) solution and 55 μL of buffers containing 50 mM urea were incubated with 5 μL of test compounds (1 mM concentration) at 30°C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn (Khan et al., 2004b). Briefly, 45 μl each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 μL of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a micro plate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 μL. The results (change in absorbance per min.) were processed by using Soft Max Pro software (Molecular Device, USA). All the assays were performed at pH 8.2 (0.005 M $K_2HPO_4.3H_2O$, 1 mM EDTA and 0.01 M $LiCl_2$). Percentage inhibitions were calculated from the formula $100-(OD_{testwell}/OD_{control}) \times 100$. Thiourea was used as the standard inhibitor of urease.

Oxidase inhibition assay

The Xanthine oxidase (XO) inhibition activity was carried out in phosphate buffer (0.1 M, pH 7.5). XO (0.003 unit/ well), 20 μL and test samples in 10 μL DMSO were mixed in 96-well microplate and pre-incubated for 10 min at room temp. The reaction was initiated by adding 20 µL of 0.1 mM of Xanthine, and the resulting uric acid formation was measured spectrophotometrically at 295 nm by using Molecular Devices, Spectramax 384 (Khan et al., 2006b).

Tyrosinase inhibition assay

Tyrosinase inhibition assays were performed in 96-well microplate format using SpectraMax 340 microplate reader (Molecular Devices, CA, USA) according to the method developed by hearing in 1987 (Khan et al., 2005a). Briefly, first the compounds were screened for the *o*-diphenolase inhibitory activity of tyrosinase using L-DOPA as substrate. All the active inhibitors from the preliminary screening were subjected to IC_{50} studies. Compounds were dissolved in methanol to a concentration of 2.5%. 30 units of mushroom tyrosinase (28 nM) were pre-incubated with the compounds in 50 nM Na-phosphate buffer (pH 6.8) for 10 min at 25°C. Then the L-DOPA (0.5 mM) was added to the reaction mixture and the enzyme reaction was monitored by measuring the change in absorbance at 475 nm (at 37°C) due to the formation of the DOPAchrome for 10 min. The percent inhibition of the enzyme was calculated as follows, by using MS Excel®™ 2000 (Microsoft Corp., USA) based program developed for this purpose:

Percent inhibition (%) = [B – S/B]×100

Here, the B and S are the absorbances for the blank and samples, respectively. After screening of the compounds, median inhibitory concentration (IC_{50}) was also calculated. All the studies have been carried out at least in triplicates and the results represents the mean ± S.E.M. (standard error of the mean). Kojic acid and L-mimosine were used as standard inhibitors for the tyrosinase. All the chemicals and reagents were purchased from Sigma Chem. Co., MO, and USA.

RESULTS AND DISCUSSION

Structures of all the compounds were elucidated using mass, IR, UV, 1D NMR (^{1}H and ^{13}C-NMR) and 2D NMR (HMBC, HMQC, COSY and NOESY). For example, compound 1 was isolated as colorless crystals from the chloroform soluble fraction of the methanolic extract. The molecular ion peak at *m/z* 456.3613 in the HRMS indicated its molecular formula to be $C_{30}H_{48}O_3$ (calcd. 456.3605). Absorption bands at 3600 - 3450, 3045 1650 and 890 cm^{-1} in the IR spectrum of 1 suggested the presence of a hydroxyl, a cyclopropane ring and a terminal methylene group, respectively. The ^{1}H-NMR spectrum displayed signals corresponding to five tertiary methyl groups at δ_H 0.92, 0.94, 0.95 (CH_3x2), and 1.70. A pair of doublets at δ_H 0.30 and 0.52 (J = 4.2 Hz) was indicative of a cyclopropane ring bearing two non equivalent hydrogen atoms. Broad singlets at δ_H 4.50, 4.60, 4.80 and 4.90 (1 H, each) were assigned to the terminal methylene protons. The doublet of double doublet at δ_H 3.98, doublet at δ_H 3.20 and a triplet at δ_H 4.01 were due to the protons attached to the carbons bearing hydroxyl groups. The ^{13}C-NMR (BB and DEPT) spectrum revealed the presence of five methyl, twelve methylene, six methine and seven quaternary carbons. It included the olefinic carbons at δc 152.5, 147.8, 111.4 and 110.8 and the oxygen bearing carbons at δc 80.6, 76.8 and 68.0, respectively.

Further information could be obtained from the mass spectrum which showed daughter ion at *m/z* 369.3011 ($C_{25}H_{37}O_2$) corresponding to the elimination of C_5H_9 from $[M-18]^+$ peak, which is characteristic of 4,4-dimethyl-9,19-cyclosterol. Another characteristics fragmentation involved elimination of the ring A producing an ion at *m/z* 300.3211 ($C_{21}H_{32}O$) and another peak at *m/z* 175.1523 ($C_{13}H_{19}$), the latter arising from the loss of the $C_8H_{15}O$ side chain from the fragment at *m/z* 300.3211. The fragmentation pattern was characteristic of cycloartane type triterpene with one hydroxyl group in the side chain and the other two hydroxyl groups in ring A. In the ^{1}H-^{1}H homonuclear chemical shift correlation spectrum (COSY-45°), the comparatively up field proton at δ_H 3.20 showed connectivity with only one proton at δ_H 3.98. It could be assigned to C-3 on biogenetic grounds. The large coupling ($J_{ax,ax}$ = 11.8 Hz) allowed us to assigned β and equatorial orientation to the hydroxyl group at C-3. The proton at δ_H 3.98 showed connectivity to H-3 at δ_H 3.20 and also with two further protons. It could be assigned to hydroxyl bearing C-2 in β and axial orientation, based on larger coupling constants (11.8, 8.4, 4.3 Hz). The possible

location of the hydroxyl group in the side chain were either at C-22 or C-24, the latter could be eliminated by HMBC experiments where the signal at δ_H 4.01 showed ^2J coupling with C-20 at δc 152.5 and ^3J interaction with C-21 at δc 110.8, respectively. The presence of a double bond at C-25 was indicated by the absence of the characteristic 6H doublet of an isopropyl group and the presence of vinyl methyl singlet at δ_H 1.70. The HMBC and spin decoupling combined with nOe difference spectroscopy allowed complete assignment of the stereochemistry of 1 (Khan et al., 2004a). The cyclopropane protons showed nOe`s with signals at $\delta_H$ 0.95 ($H_3$-18) and 0.92 ($H_3$-29) (irradiation of H-19$\beta$ at $\delta_H$ 0.30) and to H-2$\beta$ and $H_3$-18 (irradiation of H-19$\alpha$ at $\delta_H$ 0.52). The $H_3$-18 showed nOe`s with H-19β and H-19α as well as H-22β. The relative configuration at C-22 was concluded to be *R* by comparing the ^{13}C-chemical shift of δc 76.8 for C-22 which appeared more up field than the corresponding *S* epimer (Khan et al., 2004a). Thus, the structure of 1 was concluded to be (*22R*)-cycloart-20, 25-dien-2α, 3β, 22α triol. All other compounds isolated from genus *Amberboa* were characterized similarly as compound 1 using useful inhibitors for nervous system disorders; particularly, spectroscopic techniques. All the compounds are classified as cycloartanes, sesquiterpenes, flavonoids, fatty acids, lignans, sterols and other compounds. The source and biological activities of all these compounds are mentioned below.

Cycloartane

The chemical work on genus *Amberboa* started in 1954 when the presence of alkaloid was reported in *Amberboa glauca* by Zolotniktskaya, 1954). Phytochemical investigation of the methanol extract of *A. ramosa* afforded eleven cycloartane type triterpenoids and their structures were established as (*22R*)-cycloart-20,25-dien-2α,3β,22α -triol (1), (22*R*)-cycloart-23-ene-3β,22α,25-triol (2), cycloartenol (3), cycloart-23-ene-3β,25-diol (4), cycloart-20-ene-3β,25-diol (5), cycloart-25-ene-3β,(22*R*)22-diol (6), cycloart-24,25-diene-3β,21,22,23-tetraol (7), (23*R*)-5α-cycloart-24-ene-3β,21,23-triol (8), (24*R*)-cycloartane-3β,30-diol (9), cycloart-5-ene-3β,25-diol (10) and cycloartane-3β,24b,25-triol (11) through spectroscopic studies including 2D-NMR (Khan et al., 2006a; 2004a; 2005b; Akhtar et al., 1993). Compounds (1- 8) displayed inhibitory potential against the enzyme tyrosinase. Among them compound 7 was found to be the most potent (1.32 μM) when compared with the standard tyrosinase inhibitors Kojic acid (16.67 μM) and L-mimosine (3.68 μM). These are potential candidates for the treatment of melanin biosynthesis-related skin diseases (Khan et al., 2006a).

Tyrosinase (EC 1.14.18.1) is a multifunctional copper-containing enzyme widely distributed in plants and animals. It catalyzes the oxidation of monophenols, *o*-diphenols, and *o*-quinones. Tyrosinase is known to be a key enzyme for melanin biosynthesis in plants and animals. Tyrosinase inhibitors therefore can be clinically useful for the treatment of some dermatologic disorders associated with melanin hyperpigmentation. They also find use in cosmetics for whitening and depigmentation after sunburn. It has recently been shown that other factors such as metal ions and the TRP-1 and TRP-2 enzymes also contribute to the production of melanin. However, tyrosinase plays a critical regulatory role in melanin biosynthesis.

Therefore many tyrosinase inhibitors that suppress melanogenesis have been actively studied with the aim of developing preparations for the treatment of hyperpigmentation. Compounds 1 and 2 showed strong to moderate inhibitory activity against tyrosinase. Compounds 1 and 2 also displayed inhibitory potential against butyrylcholinesterase with the IC_{50} of 39.9 ± 0.6 μM and 299.1 ± 2.0 μM, respectively. Butyrylcholinesterase (BChE, EC 3.1.1.8) inhibition may be an effective tool for the treatment of *Alzheimer's* disease (AD) and related dementias. These inhibitors may act as potential leads in the discovery of clinically by reducing memory deficiency in *Alzheimer's* disease patients by potentiating and affecting the cholinergic transmission process (Khan et al., 2004a).

Cycloartanes are the triterpenes containing a 9,19-cyclolanostane skeleton. The initial compound of this series – cycloartenol is the key intermediate in the biosynthesis of cholesterol and other phytosteriods in algae and green plants (Abe et al., 1993). In view of this, cycloartane triterpenes and their glycosides are found to be widely distributed throughout the plant kingdom (Hostettman et al., 1995; Isaev et al., 1989). In recent years, cycloartanes have attracted interest not only as substances promoting our understanding to the pathways of the biosynthesis of phytosteroids and the discovery of new ones but, also as compounds possessing a broad spectrum of biological activity (Isaev et al., 1989). Cycloartanes and their glycosides possess wide ranging biological and pharmacological properties. Plants producing cycloartanes are also widely used in the traditional medicine. *Cimicifuga* and *Astragalus*, famous for their cycloartane constituents, have long been used in folk medicine for the treatment of various diseases (Isaev et al., 1985; 1989). Astragaloside II possesses anti-cancer activity against colon cancer (SW-200) and leukemia CCRF-CEM and HL-601 cell lines (Abdollah et al., 1993). Astragalozid, a preparation, which consists of combination of cycloartane glycosides from *Astragalus sieversianus* Pall., has shown hypochlosteremic activity, being capable of normalizing the lipid metabolism and improving the cardiac activity of animals in experimental endogenous hypercholesteremia. Astragalozid also possesses diuretic and hypotensive activity and can

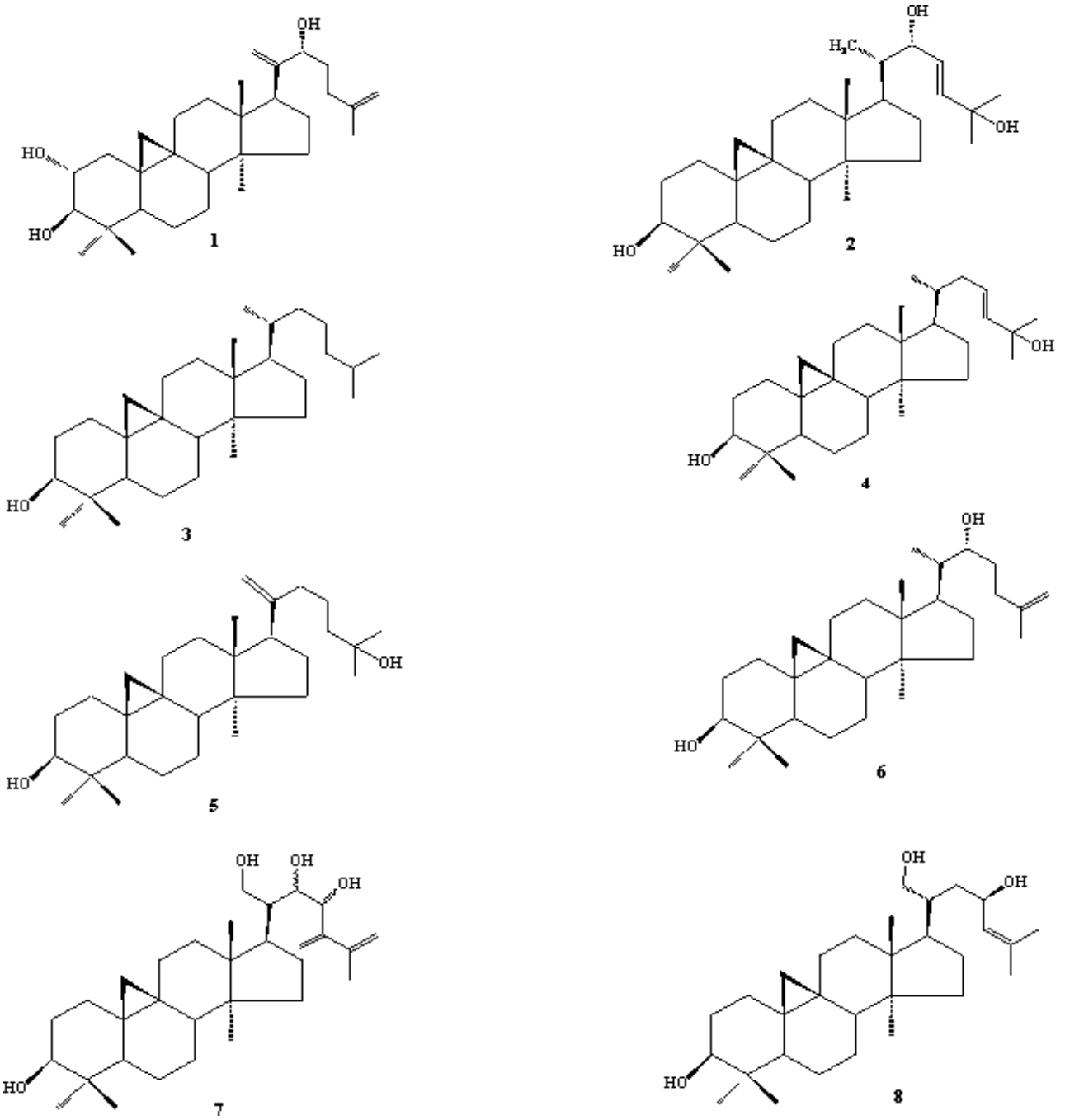
prevent the appearance of experimental gastric ulcer (Isaev et al., 1985; 1989).

Sesquiterpenes

In 1967, Gonzales et al. isolated sesquiterpene lactones amberboin (12) and grosshemin (13) from *Amberboa Lippi* (Gonzales et al., 1967). As a result of further study the same group of workers (Breton et al., 1968; Bermejo et al., 1969; Gonzalez et al., 1970) determined the structure of grosshemin and pointed out that both amberboin and grosshemin were epimeric at C_{11}. In the subsequent year two further sesquiterpene lactones lipidiol (14) and isolipidiol (15) were also isolated by them from the same species. In 1973 Gonzales et al. isolated sesquiterpene lactones muricatin (16) from *Amberboa muricata*, together with isolipidiol (15), cynaropicrin (17) and deacylcynaropicrin (18) (Gonzalez et al., 1973). In 1978, Gonzales and co-workers communicated that, amberboin; lipidiol and grosshemin possess cytostatic properties (Gonzalez et al., 1978). They suggested that α-methylene-λ-lactone group in the molecule is essential for cytotoxic activity. In the following year, Khafagy et al. isolated a sesquiterpene lactone with a α-exo-methylene and primary hydroxyl groups from the ether extract of *Amberboa tubuliflora* (Khafagy et al., 1979). It showed antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Karryev et al. in 1981 reported some biologically active compounds from *Amberboa turanica* (Karryev et al., 1981). In 1984, Omar et al. communicated the isolation of cynaropicrin (17) and deacylcynaropicrin (18) from the aerial parts of *A. tubuliflora* (Omar et al., 1983), while Harrison and Kulshreshtha in the same year reported the presence of cynaropicrin (17) in *A. ramose* (Harrison and Kulshreshtha, 1984). They also reported that cytotoxic activity of plant appeared mainly due to the presence of cynaropicrin. Ahmed and co-workers investigated the aerial parts of *A. tubulitlora* and isolated a new sesquiterpene lactone, tetrahydrozaluzanin (19) along with aguerin-β (20), cynaropicrin (17), and deacylcynaropicrin (18) (Ahmed et al., 1990).

Phytochemical investigation on the chemical constituents of *A. ramosa* resulted in isolation of three new (21-23) (Khan et al., 2004b,c) and ten (24-31) known sesquiterpenoids such as 8α-hydroxy-11α-methyl-1αH,5αH,6βH,7αH,11βH-guai-10(14),4(15)-dien-6,12-olide (24), 3β,8α-dihydroxy-11α-methyl-1αH,5αH,6βH,7αH,11βH-guai-10(14),4 (15)-dien-6,12-olide (25), 3β,4α,8α-trihydroxy-4β-(hydroxymethyl)-1αH,5αH,6βH,7αH-guai-10(14),11(13)-dien-6,12-olide (26), 3β,4α,8α-trihydroxy-4β-(chloromethyl)-1αH,5αH,6βH,7αH-guai-10(14),11(13)-dien-6,12-olide (27), 3β,α,dihydroxy-4β-(hydroxymethyl)-1αH,5αH,6βH,7αH-guai-10(14),11(13)-dien-6,12-olide (28), 3β,4α-dihydroxy-4β-(chloromethyl)-8α-(4-hydroxymethacrylate)-1αH,5αH, 6βH,7αH-guai-10(14),11 (13)-dien-6,12-olide (29), 3,4-epoxyguaia-1(10), 11(13)-dien-6, 12-olide (30) and saussureolide (31) and their structures were elucidated on the basis of spectral analysis (Khan et al., 2005b,c; 2004a). Compounds 21 and 22 displayed promising inhibitory potential against enzyme urease in a concentration-dependent fashion. Activity of ureases (E.C. 3.5.1.5) has been shown to be an important virulence determinant in the pathogenesis of many clinical conditions, which is detrimental for human and animal health as well as for agriculture. Urease is directly involved in the formation of infection stones and contributes to the pathogenesis of urolithiasis, pyelonephritis, ammonia and hepatic encephalopathy, hepatic coma, urinary catheter encrustation. It is also known to be a major cause of pathologies, induced by *Helicobacter pylori* (HP), which allows bacteria to survive at low pH of the stomach during colonization and, therefore, plays an important role in the pathogenesis of gastric and peptic ulcer (including cancer) (Khan et al., 2004b). Compounds 24-29 of them showed inhibitory potential against butyrylcholinesterase. Compounds 24 and 29 displayed weak (78.5 ± 0.06 μM) and strong (15.0± 0.05 μM) inhibitory potential, respectively, against BChE. Further, it was found that, the chlorine containing compounds 27 and 29 are the most active inhibitor of BChE; the latter is more potent than former due to the presence of hydroxyl and carbonyl functionalities in the side chain. The gradual increase in inhibitory activity of compounds 24, 25, 26 and 28 may be due to the increasing number of the hydroxyl groups. These hydroxyl moieties can form H-bonding within the active site of BChE and thus, can enhance the inhibitory potential of the compounds (Khan et al., 2005c).

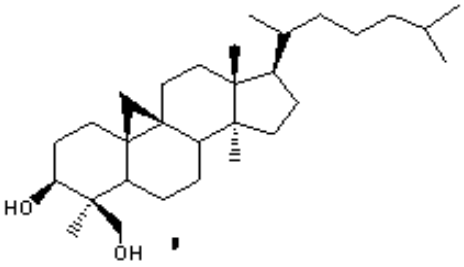
Sesquiterpens are a class of compounds, which contain a skeleton of fifteen carbons derived by the assembly of three isoprenoid units. They are mainly of plant origin, but are also present in lower animals (such as coelenterates, mollusks and arthropods) and certain fungi. The most important type of sesquiterpene is the sesquiterpene lactone in which a lactone ring makes a part of the terpene. Sesquiterpene lactones can be classified on the basis of one of the four carboxylic skeletons, pseudoguaianolides (are based upon the 5/7 carboxylic ring systemand which are C_6 or C_8 lactonized), guaianolides (based on guanine structure having C_6 or C_8 lactonization), eudesmanolides (C_6 and C_8 lactonized) and germacranolides (C_6 or C_8 lactonized and symmetrical about the C_2-C_7 axis). Apart from these four major skeletons, there are several other minor skeleton forms of sesquiterpene lactones (Ravanel et al., 1982). Sesquiterpene lactones inhibit the activity of thiol containting enzymes and as a result the protein synthesis (Picman, 1986). The famous sesquiterpenoid lactone artimesin is used against malaria. *Inula helenium* is a known therapeutical agent, it stimulates the intestinal secretion. Lactucin and lactucopicrin show sedative and hypoglycaemic activity (Picman, 1986). Verrucarin A has



antibacterial activity. Some sesquiterpenes like tercyclic acid A and heptelidic acid are antibiotic. There are sesquiterpens which are used as plant growth regulators, and some marine sesquiterpenes which have cardiotoxic, antimicrobial and related biological activities.

Flavonoids

A flavone, jaceosidine (32) was isolated from the aerial parts and flowers of *A. ramosa* (*V. divaricata*) while jaceosidine (32), crysoeriol (33), apigenin (34), and crysoeriol-7-O-glucoside (35) were isolated from the ethanolic extract of *A. ramosa* by Harrison and Kulshreshtha in 1984 (Harrison and Kulshreshtha, 1984). Recently, we isolated a new flavonol glycoside, 7,4′-dihydroxy-3,8-dimethoxylflavone 5-*O*-β-D-glucoside (36) along with eight known flavonoids (37-44) such as 6,4′-dihydroxy-3,5,7-trimethoxyflavone (37), 5,7-dihydroxy-4′-methoxyflavone (38), 6,3′-dihydroxy-3,5,7,4′-tetramethoxyflavone (39) from *A. ramosa* and their structures were elucidated by spectroscopic analysis



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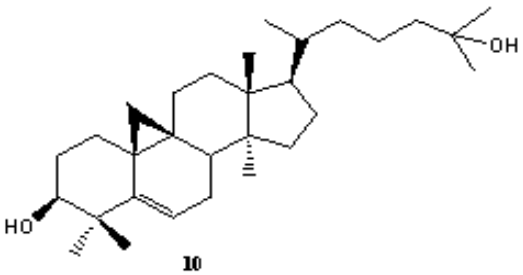

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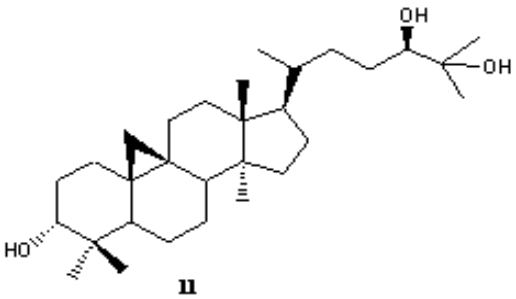

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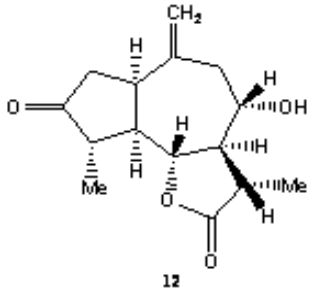

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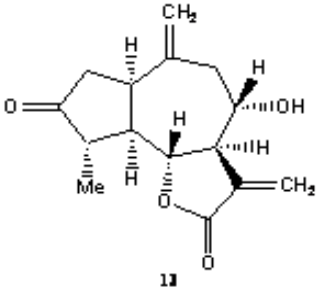

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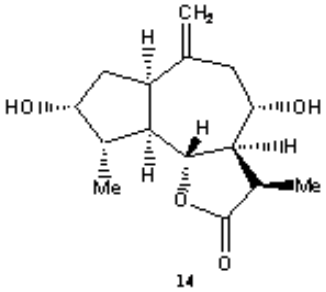

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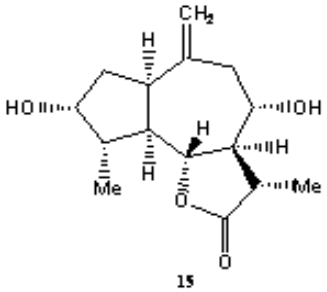

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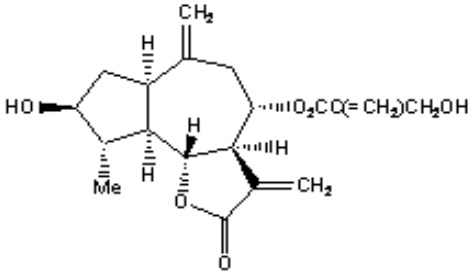

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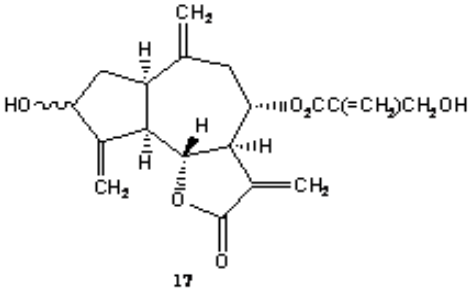

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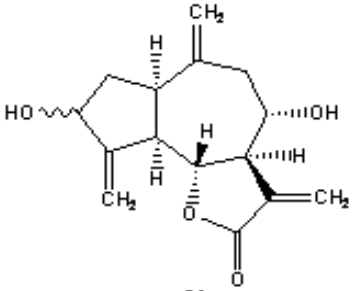

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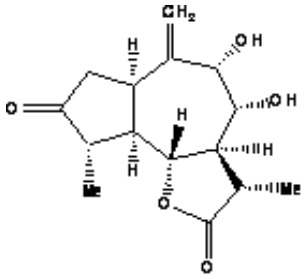

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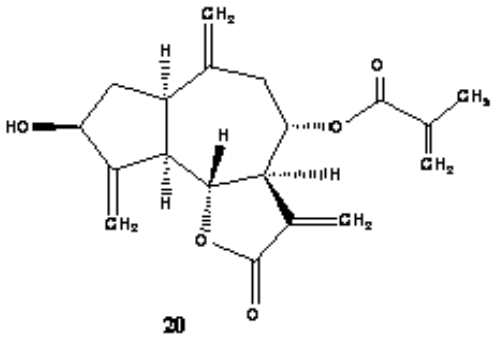

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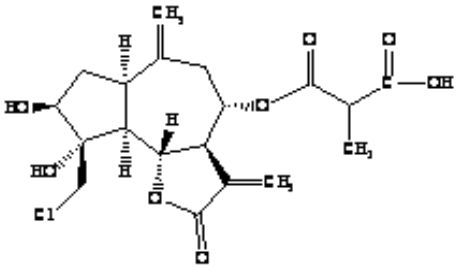

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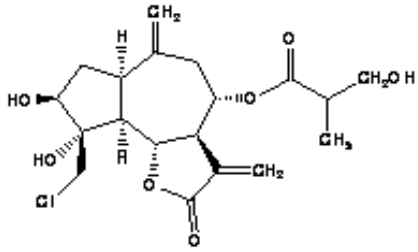

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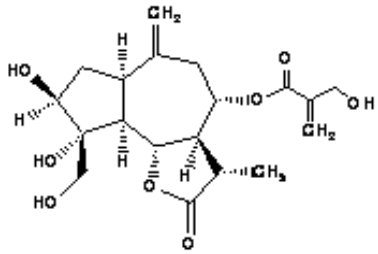

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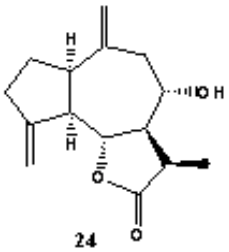

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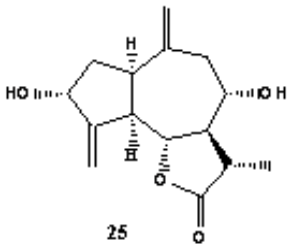

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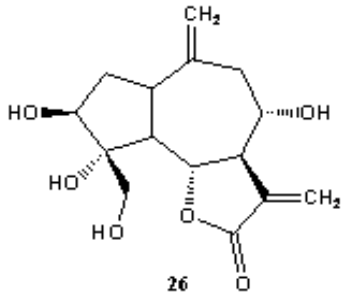

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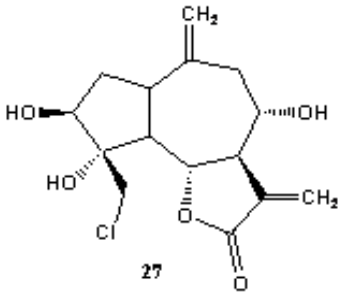

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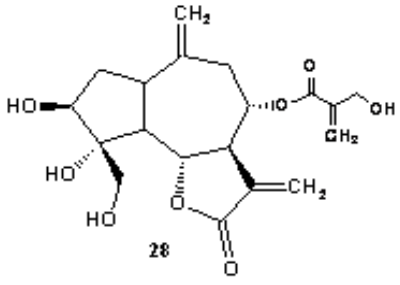


28

(Khan et al., 2006b; 2004a, b; 2005a, b). The compounds (**37-38**) displayed weak to moderate inhibition against xanthine oxidase enzyme. Xathine oxidase is a highly versatile enzyme catalyzes the hydroxylation of purines particularly conversion of xanthine to uric acid, but also the reduction of the O_2. The compounds 36-38 were tested for inhibition against xanthine oxidase and found the IC_{50} values as 408.559, 139.2 and 177.857 μM, respectively. It was evident from the results that, glucosidation of phenolic group at C-5 had a marked decreasing effect on the enzyme inhibitory action (Khan et al., 2006b).

The flavonoids, one of the most diverse and widespread group of natural products, occupy a prominent position among the natural phenols. The name “flavonoid” is derived from Greek word “flavus” (yellow). Flavonoids occur in a variety of structural forms. All contain fifteen carbon atoms in their parent nucleus and share the common structural feature of two phenyl rings linked by a three-carbon chain (diphenyl propane derivatives). The compounds possessing 1,3-diphenylpropane skeletons are regarded as chalconoids. The three-carbon chain may be formed into a third (five or six membered) ring through oxygen on one of these phenyl rings generating a tricyclic system. The tricyclic compounds possessing a five membered heterocyclic ring are referred to auronoids, whereas, those possessing a six membered heterocyclic ring are designated as flavonoids. Natural flavonoids are usually oxygenated and bear hydroxyl and/or methoxy substituents.

Various classes of flavonoids have been investigated for different physiological activities and are used in the treatment of various diseases, like capillary bleeding, increased capillary fragility, diabetes, allergic manifestation, hypertention and cold (Willaman, 1981). A number of flavonoids have anti-protozoal, anti-inflammatory and an anti HIV-I activities (Hostettman et al., 1995). Some flavonoids have very interesting activities. They have antimicrobial, anti-bacterial antileishmaniasis and anthelmintic activity (Yang and Zhou, 2007; Zheng et al, 2008; Marin et a., 2009; da Silva et al., 2008). The flavonoid glycosides have also a vital pharmacological role (Derdriguez et al., 1990). Similarly, prenylflavonol glycolside, epimedokoreanoside isolated from the *Epimedium koreamum* (Berberidaceae) showed a hypotensive activity (Pachaly et al., 1990).

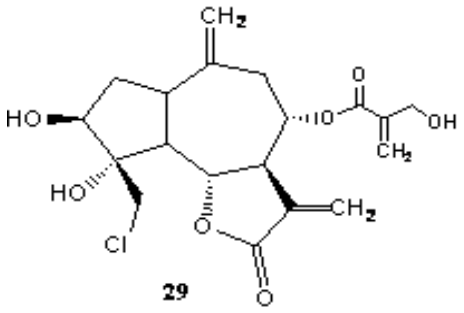
Fatty acids

Jagatap and Banergee in 1976 reported the presence of triacontane (45) in the non-saponifiable portion of the petroleum ether extract of *Amberboa divericata* (Jagatap and Banerjee, 1976) while Durdyev et al. (1984) reported the presence of carotene in *A. tubuliflora* (Durdyev and Darymova, 1983). Phytochemical investigation on *A. ramosa* resulted in isolation of two new fatty acids, methyl 2β (2*S*)-hydroxyl-7(*E*)-tritriacontenoate (46) and methyl 2β (2*S*)-*O*-β -D-galactopyranosyl-7(*E*)-tetratriacontenoate (47) along with known triacontane (45), triacontanoic acid (48) and octadecanoic acid (49) and the structures were assigned on the basis of 1D and 2D NMR techniques (Khan et al., 2005a,b). Compounds 46 and 47 showed strong to moderate inhibitory activity against tyrosinase. Compound 46, containing one –OH group at the C-2 position, exhibited highly potent (IC_{50} = 1.36 μM) inhibition against the enzyme tyrosinase compared with the standard Kojic acid (IC_{50} = 16.67 μM and L-mimosine (IC_{50} =3.68 μM). On the other hand, compound 47, which contains a D-galactopyranosyl moiety at C-2, also exhibited potent (IC_{50} = 11.68 μM) inhibition against tyrosinase compared with Kojic acid (IC_{50} = 16.67 μM), but was less potent than compound 46 and considered that this may be due to the presence of the bulky D-galactopyranosyl moiety, which possibly interferes with the entrance of the molecule into the active site of the enzyme tyrosinase. The active site of the tyrosinase contains multifunctional copper molecules that chelate mainly –OH groups. It catalyzes the oxidation of monophenols, *o*-diphenols, and *o*-quinones. It was concluded that the long-chain esters 46 and 47 can be effective inhibitors of tyrosinase enzyme and have the potential to be used for the treatment of hyperpigmentation associated with the high production of melanocytes (Khan et al., 2005a).

Fatty acids naturally occur as ester of glycerol or of some other hydroxyl compounds or as amides of long chain amines. Although, the structural diversity of this class of compounds is not as remarkable as that of other classes of natural products, this class includes many important compounds having essential functions within living systems or having important physiological activities. Natural fatty acids, both saturated and unsaturated, are straight chain compounds with an even number of carbon atoms. Despite the validity of this generalization, acids with an odd number of carbon atoms or with carbocyclic unit e.g. sterculic acid occur in nature. Acids with an unsaturated center with *cis*- (*Z*)-configuration and with the double bond in one of a limited number of preferred positions are usually alkenic compounds. Polyunsaturated acids with a methylene-interrupted arrangement of double bonds are mainly alkenic (*cis*- (*Z*)-configuration). 1,3 and 1,5-poly unsaturated acids, besides, the 1,4-unsaturated fatty acids, are also found in nature. Fatty acids rarely have any functionality apart from the carboxyl group. Various other types of functional groups occasionally observed are fluro, hydroxyl, keto and epoxy groups.

Lignans, sterols and other compounds

Tracheloside (**50**) was isolated from the ethanolic extract of *A. ramosa* by Harrison and Kulshreshtha in 1984 (Harrison and Kulshreshtha, 1984) while trachelogenin



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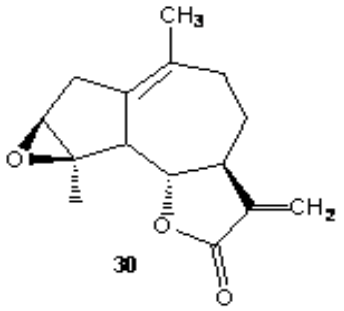

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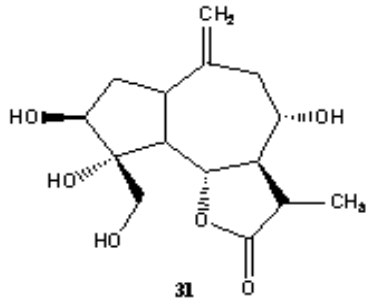

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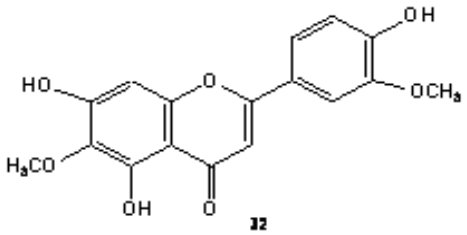

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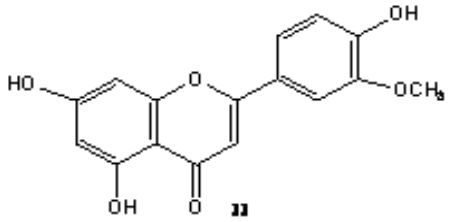

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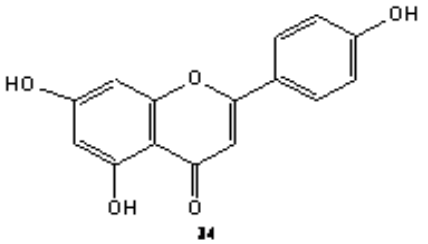

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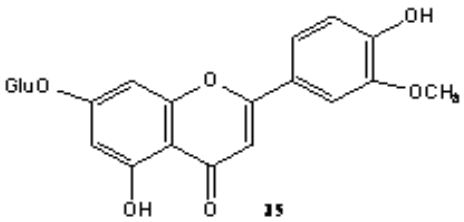

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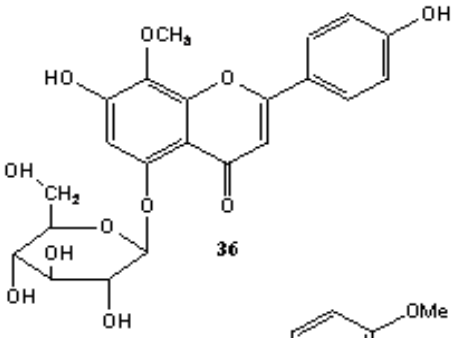

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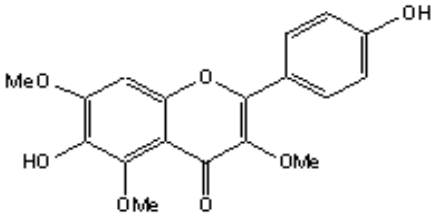

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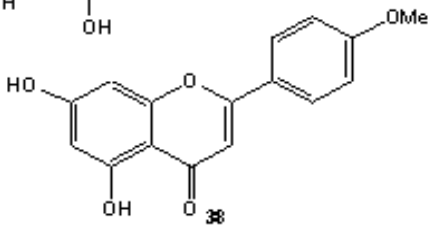

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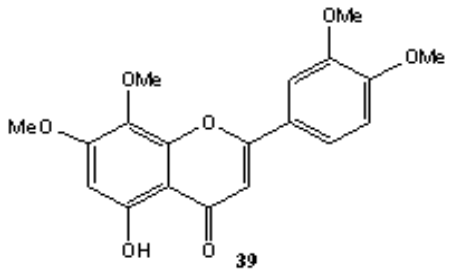

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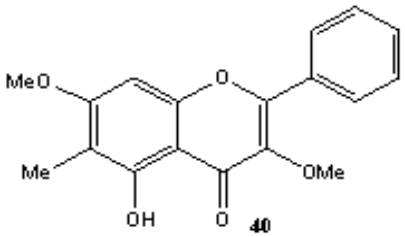

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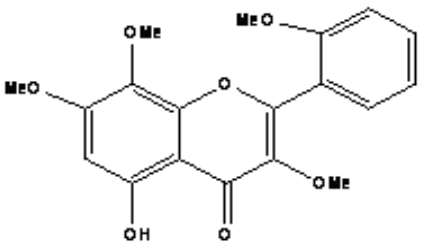

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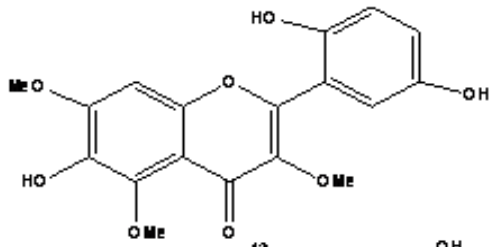

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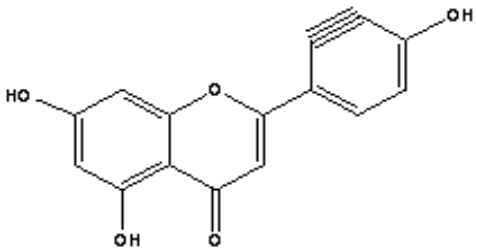

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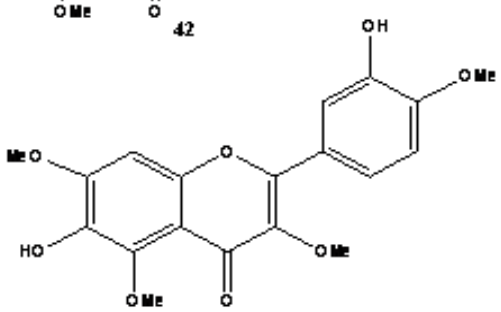

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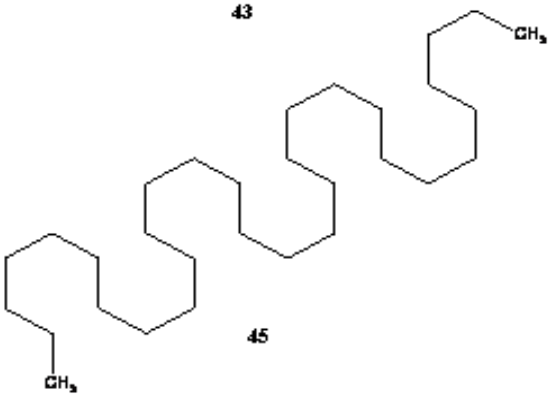

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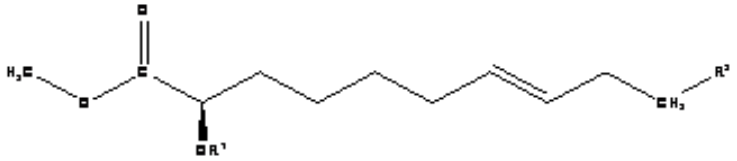

$R^1 = H$, $R^2 = C_{23}H_{47}$

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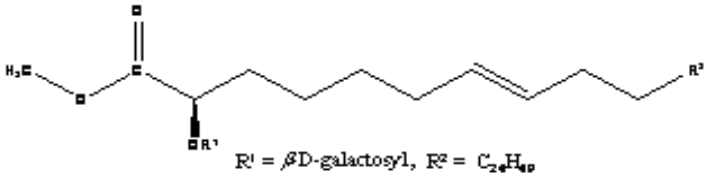

$R^1 = \beta$D-galactosyl, $R^2 = C_{24}H_{49}$

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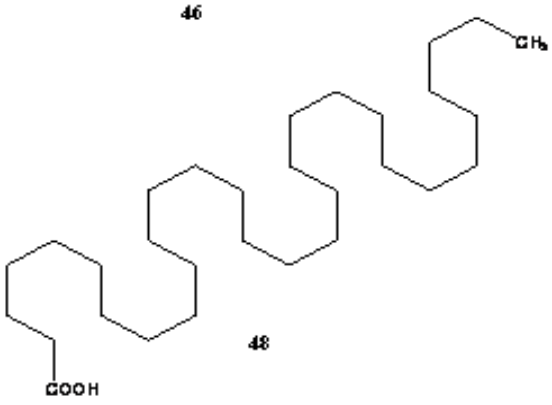

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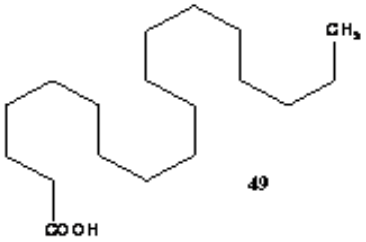

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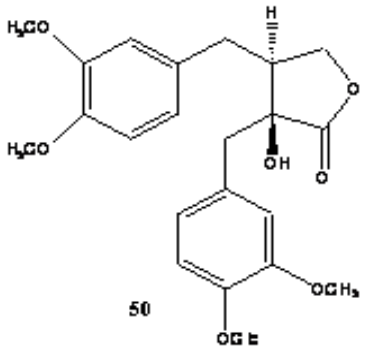

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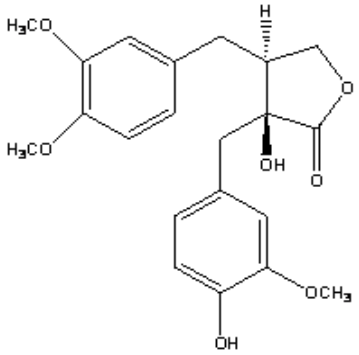

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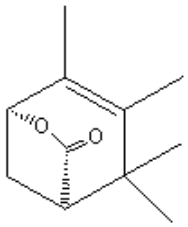

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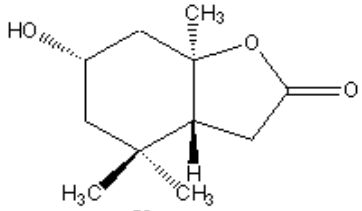

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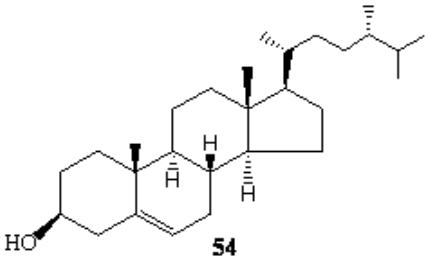

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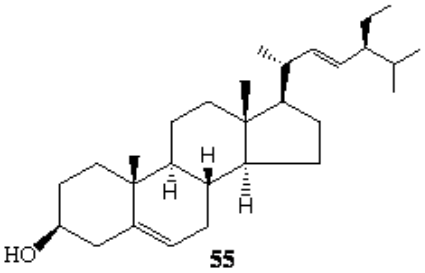

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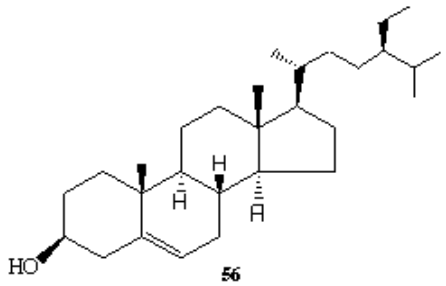

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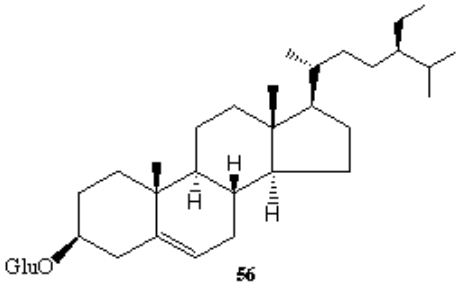
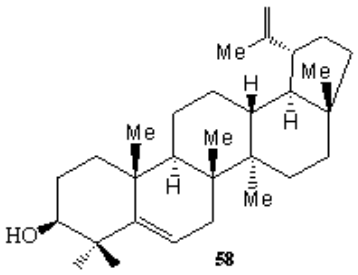


56



58

(51) along with filifolide (52) and loliolide (53) were isolated by Ahmed and co-workers [28] from the aerial parts of *A. tubulitlora* (Ahmed et al., 1990). Jagatap and Banergee in 1976 reported the presence of campesterol (54), stigmasterol (55) and sitosterol (56) in the non-saponifiable portion of the petroleum ether extract of *A. divericata* (Jagatap and Banerjee, 1976). β-sitosterol (56), and β-sitosterol-β-D-glucoside (57) were isolated from the ethanolic extract of *A. ramosa* by Harrison and Kulshreshtha in 1984 (Harrison and Kulshreshtha, 1984). Rojatkar et al. isolated lupeol (58) along with cynaropicrin (17) from the acetone extract of *A. divericata* and established their structures by chemical transformation and spectral methods. Compounds 54-58 are terpenoids which is a class of compounds characteristic of some families of terrestrial plants and microorganisms.

Terpenes comprise primary and secondary metabolites which derived from the five carbon isoprene entity. Combination and modifications of these isoprene units lead to a multitude of diverse structures with different chemical, biological and commercial applications.

Conclusion

Natural substances have been used in many industries and their commercial applications are expanded every year. However, their applicability as pharmaceutical are not well discussed. Phytomedicines are a major component of traditional system of healing in developing countries, which have been an integral part of their history and culture. Besides widespread use of botanicals as medicinal products in developing countries, such products are becoming part of the integrative healthcare system of industrialized nations, known as complementary and alternative system of medicines.

The increasing faith of the population on herbal treatment has given birth to the urge to explore natural wealth so that potent therapeutic natural compounds may be utilized for health treatment. Identification of pharmaceutical potential of natural compounds is a growing field and use of plants makes a new approach to be able to develop more commercial applications. So far, a large number of natural metabolites have been isolated but very limited numbers have been used as a pharmaceutics and further researches are needed to get more pharmaceutical to apply them for the human health promotion. For such purpose, an effort has been taken to review the secondary metabolites isolated from *Amberboa.* These metabolites are classified as cycloartanes, flavonoids, sesquiterpenes, fatty acids, lignans and sterols and have been intensively investigated and studied their biological activities. These metabolites or their modification may lead to the useful pharmaceutical apply for the human health promotion.

Furthermore, the inactive plant extracts may be subjected to chemical diversification of their components to increase the activity. The transformation of chemical groups in natural products into rare chemical groups is possible which is rarely produced by secondary metabolism. Therefore, biosynthesis machinery can be complemented to produce a whole range of new semi-synthetic compounds in one step which may become an alternative source of compounds to feed the discovery process for new interesting compounds. The study of alternative mechanisms of infection, prevention and treatment is essential. Plant products furthermore may be structurally modified to increase their activity.

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