Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 3, 2012

Research Article

NATURAL ANTIOXIDANT (FLAVONE GLYCOSIDE) FROM *EMILIA SONCHIFOLIA* DC. AND ITS POTENTIAL ACTIVITY

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Received: 14 Dec 2011, Revised and Accepted: 11 Feb 2012

ABSTRACT

This paper deals with the significance and importance of medicinal plants. New flavone glycoside A, m. p. 222-224ºC, m. f. $C_{34}H_{42}O_{20}$, [M^+] 770 (FABMS) has been isolated from the stems of *Emilia sonchifolia* DC. along with three known compounds Kaempferol 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, (B) and Mearnsetin-3-*O*-α-L-rhamnopyranoside (C) Gehuain **(**isoflavone**)** (D) . The compound **A** was characterized as 5, 7, 8-trihydroxy-6, 4'-dimethoxy flavone-7-O-α-L-rhamnopyranosyl-1→4)-O-β-D-xylopyranosyl-(1→4)-galactopyranoside by various chemical degradations and spectral analysis. Compound was subjected to antioxidant, antibacterial activity. Compound A showed potent antioxidant activity, of which the methanol and chloroform fraction demonstrated the strongest. Antioxidant activity with the IC50 value of 52.40 µg/ml and 60.15 µg/ml, respectively.

Keywords: Antioxidant and antibacterial Activity of *Emilia sonchifolia* DC., Compositae.

INTRODUCTION

Plants are a rich source of natural products. They form major parts of ingredient in almost all system of therapeutics. Most of pharmaceutical industries are conducting extensive research on plants collected from the rain forests and other places for their potential medicinal values. Modern allopathic system of medicine is also based on plants and herbs. Medicinal plants are relevant in both developing and developed nations of the world as sources of drugs or herbal extracts for various chemotherapeutic purposes continue to play a dominant role in maintenance of human health since antiquities. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs of the pharmaceutical industry. In the continuation of this strategy of new drug discovery we have studied only the aerial parts of the plant for their antibacterial and antioxidant properties.

Emilia sonchifolia DC.[1-2] belongs to family Compositae. It is commonly known as ′Hiranakhuri′ in Hindi. It is found throughout in India, Ceylon, most tropical and subtropical regions. It is a glabrous scabrid or puberulous slender herb, 30-40 cm. high. It is edible and used as a salad plant before flowering. The stem-leaves are cooked and eaten as vegetable. The plant is sudorific. A decoction of it is used as febrifuge in infantile tympanites and in bowel complaints. Its root is used for diarrhoea. The juice of fresh leaves is used for sore ears, sore eyes and night-blindness. Earlier workers[3-5] have reported various constituents from this plant. In the present paper, we report the isolation and structural elucidation of a new flavone glycoside-5, 7, 8-trihydroxy-6, 4′-dimethoxy flavone-7-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→4)-galactopyranoside (A) along with three known compounds Kaempferol -3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, (B) and Mearnsetin-3-O-α-L-rhamnopyranoside (C) Gehuain **(**isoflavone**)** (D) from methanolic extract of the stems of this plant.

MATERIALS AND METHODS

General Experimental Procedure

All the melting points were determined on a thermoelectrically melting point apparatus and are uncorrected. UV Spectra was determined on Shimadzu-120 double beam spectrophotometer in MeOH. The IR Spectra were recorded on Shimadzu FTIR-8400 spectrophotometer in KBr disc. ^{1}H-NMR Spectra were recorded on Varian XL 400 MHz spectrometer in $CDCl_3$ using T M S as internal standard. ^{13}C-NMR Spectra were recorded on Varian XL 100 MHz spectrometer using $CDCl_3$ The FAB mass spectra were recorded on a JEOL SX -102/DA-6000 Mass Spectrometer /Data System using Argon/Xenon (6kv) as the FAB gas. Thin Layer Chromatography on silica gel G and column chromatography on silica gel were used. All solvents, chemicals and reagents were of analytical grade.

Plant Material

The stems of *Emilia sonchifolia* DC. were procured from Satbhaiya plant suplier katra bazar Sagar and were taxonomically authenticated by the department of Botany Dr. H. S. Gour University Sagar (M.P.) India.

Extraction and Isolation

Air dried and powdered flowers (6.10 kg) of the plant were extracted with rectified spirit in Soxhlet extractor for three days. The flowers were successively extracted with methanol for five days. The MeOH soluble fraction of the plant was concentrated under reduced pressure, to yield a light brown viscous mass (2.8 gm) which was subjected to TLC examination over silica gel-G using n BAW (4:1:5) as solvent and I_2 vapors as visualizing agent, showed three spots, indicating it to be mixture of three compounds A, B and C . These compounds were separated and purified by column chromatography over silica gel using $CHCl_3$

MeOH in various proportions. After removal of the solvent and crystallization from ether, above eluates yielded compound A (1.72 gm.), compound B (0.64 gm.) and compound C (0.55 gm) respectively.

Study of Compound A

It had m. p. 211-212ºC, m. f. $C_{34}H_{42}O_{20}$, [M^+] 770 (FABMS) found (%); C 53.01, H 5.47, calcd for m. f. $C_{34}H_{42}O_{20}$, (%); C 52.98, H 5.45, UV MeOH λ_{max} (nm); 273, 322 (+$AlCl_3$), 302, 350 (sh) (+$AlCl_3$ / HCl), 284, 354 (+NaOAC); 247, 286 (+NaOMe); 283, 324. IR (KBr) ν_{max} (cm^{-1}) 3484, 3210, 2930, 1738, 1650, 1610, 1432, 1325, 1085, 810. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.05 (1H, s, H-3), 7.33 (2H, d, J 8.4 Hz, H-2′, 6′), 6.80 (2H, d, J 8.4, Hz, H-3′, 5′), 11.87 (1H, s, OH-5), 12.05 (1H, s, OH-8), 5.80 (1H, d, J 7.2 Hz, H-1′′), 4.40 (1H, dd, J 8.4, 7.5, Hz, H-2′′), 4.35 (1H, dd, J 8.2, 8.1 Hz, H-3′′), 3.91 (1H, dd, J 8.2, 8.2 Hz, H-4′′), 3.98 (1H, dd, J 8.0, 6.4 Hz, H-5′′), 4.20 (2H, dd, J 6.12, 10.2 Hz, H-6′′), 5.02 (1H, d, J 6.4 Hz, H-1′′′), 3.78-3.92 (3H, m, H-2′′′, H-3′′′, H-4′′′), 4.25 (2H, dd, J 6.12, 11.5 Hz, H-5′′′), 5.23(1H, br, s, H-1′′′′), 3.96-4.50 (4H, m, H-2′′′′, 3′′′′, 4′′′′, 5′′′′), 1.56 (3H, d, J 5.3 Hz, Rham-6′′′′), ^{13}C-NMR (90 MHz, $CDCl_3$), δ 162.6 (C-2), δ 107.6 (C-3), δ 175.9 (C-4), δ 159.5 (C-5), δ 133.1 (C-6), δ 162.7 (C-7), δ 95.0 (C-8), δ 154.4 (C-9), δ 103.6 (C-10), δ 120.9 (C-1′), δ 128.6(C-2′), δ 115.3 (C-3′), δ 162.9 (C-4′), δ 117.0 (C-5′), δ 128.5 (C-6′), δ 102.01 (C-1′′), δ 84.6 (C-2′′), δ 78.5 (C-3′′), δ 70.8 (C-4′′), δ 76.2 (C-5′′), δ 65.3 (C-6′′), δ 105.9

(C-1′′′), δ 76.8 (C-2′′′), δ 78.2 (C-3′′′), δ 73.5 (C-4′′′), δ 75.3 (C-5′′′), δ 99.4 (C-1′′′′), δ 85.0 (C-2′′′′), δ 75.6 (C-3′′′′), δ 70.1 (C-4′′′′), δ 76.0 (C-5′′′′), δ 67.2 (C-6′′′′),. MS (FABMS) m/z 770 [M+] , 624 [M+ - rhamnose], 492 [M+ rhamnose-xylose], 330 [aglycone].

Acid hydrolysis of Compound A

Compound **A** (75 mg) was dissolved in ethanol (15 ml) and refluxed with 15 ml of 10% H_2SO_4 on water bath for 7 hrs. The contents were concentrated and allowed to cool and residue was extracted with diethyl ether. The ether layer was washed with water and the residue was chromatographed over silica gel using $CHCl_3$:MeOH (6:4) as solvent to give compound A-1 which was identified as 5,7,8-trihydroxy-6,4′-dimethoxy-flavone by comparison of its spectral data with reported literature values. The aqueous hydrolysate obtained after hydrolysis was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off, the filtrate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) solvent system and sugars were identified as D-galactose (R_f 0.19), D-xylose (R_f 0.26), and L-rhamnose (R_f 0.36).

Permethylation of Compound A

Compound A (45 mg) was refluxed with MeI (5 ml) and Ag_2O (25 mg) in DMF (25 ml) for 4 hrs and then filtered. The filterate was hydrolysed with 10% ethanolic H_2SO_4 for 6 hrs. to give methylated aglycone, identified as 7-hydroxy- 5,6,8,4′-tetramethoxy flavone and methylated sugars which were identified as 2,3,4-tri-O-methyl-L-rhamnose, 2,3,4-tri-O-methyl-D-xylose and 2,3,4,6-tetra-O-methyl-D-galactose

Enzymatic hydrolysis of Compound A

The compound **A** (40 mg) was dissolved in MeOH (25 ml) and hydrolysed with equal volume of takadiastase enzyme. The reaction mixture was allowed to stay at room temperature for two days and filtered. The proaglycone and hydrolysate were studied separately.

The hydrolysate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) as solvent systems and aniline hydrogen phthalate as spraying reagent, showed the presence of L-rhamnose (R_f 0.36). The proaglycone was dissolved in MeOH (20 ml) and hydrolysed with equal volume of almond emulsin at room temperature as usual procedure, yielded aglycone identified as 5,7,8-trihydroxy-6,4′-dimethoxy-flavone and sugars were identified as D-galactose (R_f 0.19) and D-xylose (R_f 0.26) (Co-PC).

Study of Compound A-1

It had m.f. $C_{17}H_{14}O_7$, m.p. 198-200ºC, [M+] 330 (FABMS), found (%); C 61.83, H. 4.26, calcd for m.f. $C_{17}H_{14}O_7$, (%) C 61.81, H 4.24. UV MeOH λ_{max} (nm); 280, 362, (+$AlCl_3$); 308, 352, (+$AlCl_3$ / HCl); 285, 355 (+NaOAc); 248, 286, (+NaOMe); 283, 334. IR (KBr) ν_{max} (cm^{-1}); 3250, 2955, 1714, 1635, 1600, 1540, 1428, 1370, 1255, 1082, 810. ^{1}H-NMR (300 MHz, $CDCl_3$); δ 7.24 (1H, s, H-3), 7.38 (2H, d, J 8.5 Hz, H-2′, 6′), 7.10 (2H, d, J 8.5, Hz, H-3′, 5′), 12.64 (s, OH-5), 12.08 (s, OH-8), 3.9 (3H, s, OMe-6), 4.12 (3H, s, OMe-4′). ^{13}C-NMR (90 MHz, $CDCl_3$); δ 161.4 (C-2), δ 106.8 (C-3), δ 176.5(C-4), δ 158.2 (C-5), δ 130.6 (C-6), δ 162.3 (C-7), δ 94.6 (C-8), δ 154.5 (C-9), δ 103.9 (C-10), δ 120.4 (C-1′), δ 128.6 (C-2′), δ 117.0 (C-3′), δ 152.0 (C-4′), δ 116.1 (C-5′), δ 128.7 (C-6′), MS (FABMS) m/z 330 [M+] .

RESULTS

Compound **A** showed R_f value of 0.59 & showed R_f value of 0.55 in $CHCl_3$: MeOH (95:5) solvent system. It gave orangish yellow colour with 10% methanolic sulfuric acid and pink colourwith Shinoda confirmed the presence of flavone and also showed positive Molisch test with formation of violet ring along with three known compounds Kaempferol 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside,(B) and Mearnsetin-3-O-α-L-rhamnopyranoside (C) Gehuain **(**isoflavone**)** (D) by comparisons of their spectral data (U.V, I.R, NMR and MS). All compounds showed moderate activity against Gram-positive, Gram-negative bacteria and fungi are given in **Table-I**. Compound **A** showed highest activity against gram positive bacteria *Staphylococcus aureus* and showed less activity against *Escherichia coli.* The A was found to be highly active against *Aspergillus niger* and *Penicillium digitatum*. Thus on the basis of above results it was concluded that above compound may be potentially used as diseases caused of these microorganisms.

DISCUSSION

Compound **A** had m.f. $C_{34}H_{42}O_{20}$, m.p. 211-212ºC, [M+] 770 (FABMS). It gave Molisch[6] and Schinoda test[7] showing its flavonoidal glycosidic nature. The compound also responded to neutral ferric chloride test. The UV and IR spectral data also shows the nature of flavone. The IR spectrum showed strong absorptions at 3484 (-OH), 2930 (-CH saturated), 1672 (>C=O), 1610 (aromatic ring). In its UV spectrum, absorption bands at 272 nm and 322 nm indicating its isoflavonoid skeleton. Two bathochromic shifts of 26 nm and 44 nm in bands I on addition of $AlCl_3$ and $AlCl_3$ + HCl relative to methanol confirm the presence of -OH group at C-5 and C-6 position. A bathochromic shift of 15 nm in bands I with NaOMe showed the presence of -OH group at C-4' position in compound **A**[8, 9]. In ^{1}H-NMR spectrum of compound A, two singlets at δ 7.43 and δ 6.85 were assigned to H-2′, 6′ and H-3′, 5′ respectively. One sharp singlet at δ 7.6 were assigned to H-2 proton. A singlet at δ 3.78 confirmed the presence OMe group at C-8 position. The anomeric proton signals at δ 5.24 (1H, d, J 7.4 Hz, H-1′′), δ 6.40 (1H, d, J 7.4 Hz, H-1′′′) and δ 5.42 (1H, d, J 6.4 Hz, H-1′′′′) were assigned to H-1′′ of L-rhamnose, H-1′′′ of D-galactose, H-1′′′′ of D-xylose' and Characteristics ions appeared at m/z 770 [M+], MS (FABMS) m/z 756 [M+], 610 [M+ - rhamnose], 478 [M+ rhamnose-xylose], 330 [aglycone].

Acid hydrolysis of compound **A** with ethanolic 10% H_2SO_4 yielded aglycone **A-1**, m.p. 265-266ºC, m.f. $C_{17}H_{14}O_7$, [M+] 330 (FABMS) which was identified as 5, 7, 8-trihydroxy-6, 4′-dimethoxy flavone by comparison its spectral data with reported literature values[10-11]. The aqueous hydrolysate after the removal of aglycone, was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to TLC and paper chromatography examination[12-13] and the sugars were identified as D-galactose (R_f 0.19), D-xylose (R_f 0.26), and L-rhamnose (R_f 0.36) (Co-PC, Co-TLC). Periodate oxidation[14-15] of compound **A** confirmed that all sugars were present in the pyranose form. The glycosidic linkage is located at 7-position in aglycone [16]

The position of sugar moieties in the compound **A** were determined by permethylation[17-18] followed by acid hydrolysis which yielded methylated aglycone identified as 7-hydroxy-5, 6, 8, 4′-tetramethoxy-flavone which confirmed that hydroxy group at C-7 position of the aglycone were involved in glycosidation. The methylated sugars which were identified as 2, 3, 4, 6-tetra-*O*-methyl-D-galactose, 2,3,4-tri-*O*-methyl-D-xylose and 2, 3, 4-tri-*O*-methyl-L-rhamnose according to Petek indicating that the C-1′′′′ of L-rhamnose was linked to C-4′′′ position of xylose and C-1′′′ of D-xylose was attached with C-4′′ of D-galactose and C-1′′ of D-galactose was attached to the C-7 position of aglycone and also showed the interlinkage (1→4) between D-xylose and D-galactose. That was further confirmed by their ^{13}C-NMR spectral data. Enzymatic hydrolysis[19] of compound **A** with takadiastase enzyme liberated L-rhamnose (R_f 0.37) and proaglycone identified as 5, 7, 8-trihydroxy-6, 4′-dimethoxy-flavone-7-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-galactopyranoside that confirmed the presence of α-linkage between L-rhamnose and C-5 position of aglycone. Proaglycone on further hydrolyzed with almond emulsin liberated D-galactose (R_f 0.19), D-xylose (R_f 0.26) suggesting the presence of β-linkage between D-xylose and D-galactose as well as D-galactose and aglycone. On the basis of above evidences, the structure of compound **A**, was characterized as 5, 7, 8-trihydroxy-6,4′-dimethoxy-flavone-7-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-xylopyranosyl (1→4)-O-β-D-glactopyranoside.

Compound B yellow powder; was analysed for m. p.221-222ºC, m. f. $C_{27}H_{30}O_{15,}$ M^+ 594 (ESIMS), m/z 593 [M-H] and identified as Kaempferol 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, by comparison of its spectral data with reported literature values[20].

Compound C yellow amorphous powders, was analyzed for, m. p.261-262ºC, m. f. $C_{26}H_{26}O_{14,}$ M^+ 585 (ESIMS)., found (%) C 56.97 H

Compound D yellow amorphous, was analyzed for, m. p. 120-121ºC, m. f. $C_{26}H_{26}O_{14,}$ M^+ 585 (ESIMS), found (%) C 56.97, H 5.18 cacld for m. f. $C_{22}H_{24}O_{11}$, % C 56.89, H 5.17 . UV MeOH λ_{max} (nm); 268 (4.40),

324 (3.92) nm. IR (KBr) ν_{max} (cm^{-1}); 3400, 2923, 1641, 1520, 1491, 1372, 1279, 1187, 1039 cm^{-1} and identified as Gehuain **(**isoflavone**)** by comparison of its spectral data with reported literature values[22]**.**

Determination of Antimicrobial Activity of the Compounds

The antimicrobial activity of compound A was determined by Filter Paper Disc Diffusion Method[23]. The various bacterial species were first incubated at 45°C for 48 hrs. The sterile filter paper discs (6mm) were soaked with standard antibacterial agent and various test samples and were dried at 50°C. The disc were then placed on soft nutrient agar (2%) petri plates previously seeded with suspension of each bacterial species. The diameters of zone of inhibition were measured at 37 ± 1°C after 24 hrs.

For fungal activity Saboraud's broth media[24] with 4% agar was used for the preparation of plates and incubated with spores and mycelium suspension of fungi obtained from one week old culture. The diameter of zone of inhibition was measures at 28 ± 1°C after 48 hrs. The various results are recorded in Table 1.

Table 1: Antibacterial and antifungal activity of compounds

S. No.	Bacterial Species	Diameter of Zone of Inhibition (mm)				
		Compd A (100 %)	Compd B (100 %)	Compd C (100 %)	Compd D (100 %)	Std* (100 %)
1	*(+)S.aureus*	13.4	11.5	11.5	11.5	19.21
	(-)Escherichia coli	7.5	12.5	9.5	11.4	15.80
2	*(+)Bacillus coagulas*	6.8	16.5	12. 5	9.5	16.50
3	*(-)P.aeruginosa*	8.5	10.4	16.7	12.5	17.64
4	**Fungal Species**					Std**(100 %)
1	*Aspergillus niger*	6.4	14.5	8.4	11.5	16.8
2	*Penicillium digitatum*	10.5	16.4	7.5	2.8	21.0
3	*Trichoderma viride*	11.6	12.5	9.5	7.4	15.2

* Streptomyc for antibacterial and ** Griseofulvin for antifungal used as standard drugs

Antioxidative Activity

DPPH assay

The Hydrogen atom or electron donation ability of compound A and some pure compounds were measured from the bleaching of the purple coloured methanol solution of DPPH. This spectrophotometric assay used stable radical DPPH as a reagent[25]. 50ml of various concentrations of methanol, chloroform extracts were added to 5ml of 4mg /100ml methanol solution of DPPH. After 30 min of incubation period at room temp, the absorbances were read against a blank at 517nm. Inhibition of free radical DPPH in percent (I%) was calculated as fallows:

$$\text{Blank I \%} = [(\bar{A} \text{ Asample }) / \text{ A Blank}] \text{ X } 100$$

Where A blank is the absorbance of control reaction (Containing all reagents except test compound) and A sample is the absorbance of the test compound. Compound oncentration providing 50% inhibition (IC50) was calculated. Ascorbic Acid (AA) was used as positive control.

β-Carotene and Linoleic acid assay

Antioxidative capacity was determined by measuring the inhibition of the volatile organic compound and the conjugate diene hydroperoxides arising from linoleic acid oxidation[26]. A stock solution of β-Carotene/Linoleic acid was prepared as 0.5 mg β-Carotene was dissolved in 1ml of chloroform (HPLC grade), 2.5µl linoleic acid and 200mg Tween-40 were added. Chloroform was completely evaporated using vacuum evaporation. 100ml of distilled water saturated with O_2 was added with vigorous shaking (30min, 100rpm). 2.5 ml of reaction mixture was dispersed to test tubes and 350 µl portions of the extracts (prepared in 2g/l of methanol and chloroform extracts) were added and then emulsion system was incubated for 48 hrs at room temperature. Ascorbic acid was used as positive controls. Absorbance of the mixture was measured at 490 nm. Antioxidative capacities were compared with those of AA with same concentration and a blank consisting of only 350 µl of ethanol.

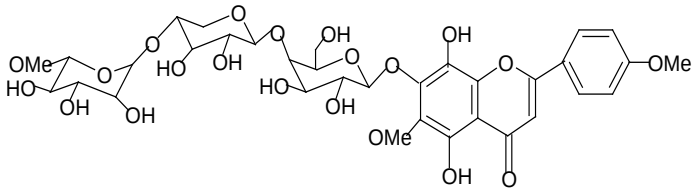
Antioxidant activity of methanol and chloroform soluble fraction of compound A tested by comparing it to the activity of known oxidant such as AA with the inhibition of DPPH radical. In addition, the effect of inhibition of lipid peroxidation of the extract was assayed using β-carotene bleaching induced linoleic acid peroxidation method. Free radicals involved in the process of lipid peroxidation play a primary role in numerous chronic diseases and are implicated in the aging process. In present study phytochemicals are recognized as scavengers of DPPH and Anti oxidant activity.

The Result of antioxidant activity

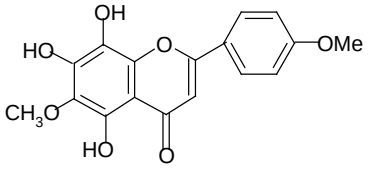
The antioxidant activity of the compound was assessed by the DPPH free radical scavenging assay as shown in table 2. Compound exhibited potential antioxidant activity. The chloroform soluble part of compound A scavenged 50% DPPH free radical at the lowest inhibitory concentration (IC50: 52.45µg/ml). The methanol soluble compound also revealed strong antioxidant activity (IC50: 60.15µg/ml). On the other hand, methanol soluble and chloroform soluble fraction of compound A showed antioxidant activity with IC50 of 60.15µg/ml and 52.45µg/ml respectively.

Table 2: IC_{50} data of test samples of Compound A, Ascorbic acid and BHT

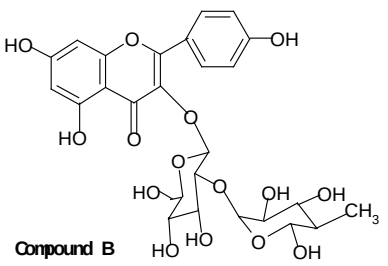
S.No.	Samples	IC_{50} (µg/ml)
1	Ascorbic acid	41.05
2	Methanolic compound A	60.15
3	Chloroform Soluble compound A	52.45



Compound (A)



Compound (A-1)



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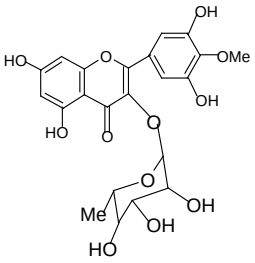
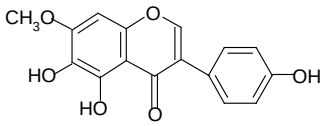


Compound C



Compound D

Fig. 1: Compounds isolated from *Emilia sonchifolia* DC.

CONCLUSIONS

Presently natural products research has an important role of the drug discovery process of the pharmaceutical industry and other research organizations. Chemical and biological research on natural products over the past two centuries has not only provided drugs for the treatment of various human ailments, but has provided the stimulus for the development of modern synthetic organic chemistry, and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents. The isolation and antimicrobial activity of 5,7,8-trihydroxy-6, 4'-dimethoxy flavone-7-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→4)-galactopyranoside from stems of Sesbania aculeata Pers is being reported for the first time and showed good potential activity.

ACKNOWLEDGEMENT

Authors are grateful to Head, RSIC, CDRI Lucknow for recording various spectral and elemental analysis and grateful to Head Department of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar (M.P.) for screening of Antioxidant and antimicrobial activity also grateful to Head Department of Chemistry, Dr. H. S. Gour University, Sagar (M.P.) India, for providing necessary Laboratory facilities.

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