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ISOLATION, CHARACTERIZATION AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF A FLAVANONE DERIVATIVE 8-HYDROXYL NARINGENIN FROM *ELEPHANTOPUS SCABER* LINN

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ABSTRACT

Elephantopus scaber Linn. is a small perennial herb belongs to the family Asteraceae found in tropical climates. The leaves and the roots are used in treating diahorrea, liver disorders, tumors, inflammations and ulcers. The phytochemical constituents of this plant indicate that it contains sesquiterpene lactones, terpenoids, deoxyelephantopin etc. Bioactivity guided fractionation of its acetone extract led to the isolation and characterization of a novel flavanone, 8- hydroxyl naringenin. The structure of the isolated compound A was elucidated on the basis of NMR spectrum and mass spectrum as well as by comparison with available data in the literature. The isolated compound A showed inhibitory effect on all 12 tested bacteria like *Bacillus cereus*, *Salmonella typhi* etc. Compound A, 8- hydroxyl naringenin was active more than the crude acetone extract of *E. scaber*.

KEYWORDS: *Elephantopus scaber*; flavanone; 8- hydroxyl naringenin; antibacterial activity; NMR.

INTRODUCTION

Medicinal plants continue to be an important therapeutic aid for alleviating ailments of humankind. According to the World Health Organization, over 80% of the world's populations rely upon such traditional plant based systems of medicine to provide them with primary health care[1]. The increasing prevalence of multidrug resistant strains of bacteria and

the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies. Adverse effects of popular antibiotics and multidrug resistant strains of pathogens have lead rapid search for new antimicrobials. Because of the long history of plants in the treatment of different human ailments, most of the herbal drugs are believed to be safer than the synthetic drugs with no side effects; therefore medicinal plants have gained more importance as possible source of alternative and effective drugs. Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. Indigenous medicinal plants play significant role of an economy of a country. Use of plants as a source of medicine has been inherited and is an important component of the health is systems in India. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment[2]. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine. Plants are known to contain a large number of biologically active compounds and they yield valuable herbal products which are often used in the treatment of a variety of conditions. Compounds, which emerged from the study of ethnobotanic extracts became important as medicines and were enabling pharmacologic tools in the elucidation of disease mechanisms[3]. Biomedicines are literally god/goddess gift, rather than developed in a laboratory. Though it is difficult to understand the actions of individual active constituents of the herbal medicine. In fact, biomedicine is ultimately about the use and actions of whole plants. A herb is not serve as a magic bullet because it is composed of several active constituents which work on different human body systems. By combining scientific research into active constituents with clinical observation and traditional knowledge of the whole plant, we can develop a rounded picture of each herb's range of medicinal uses. The isolation of active compounds should be undertaken in light of the known activity of the plant and likewise follow a guided isolation of potential principles. Thus, when the activity of fractions and compounds is inferior to the total extract or fraction, rather than invalidating the results, this should confirm the known anti-infection properties of the plant[4]. Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need, because their structures are different from those of the more studied microbial sources, and therefore their mode of action

may likely to differ [5]. *Elephantopus scaber* Linn. is a small perennial herb belongs to the family Asteraceae found in the dry or semi arid regions including central India[6,7]. The plant has been extensively used in different systems of medicine, for the treatment of various types of diseases. Traditionally the whole plant used as cardiotonic, astringent, antidote for snake bite, febrifuge and diuretic. The plant contains valuable chemicals like geracranolide dilactones, deoxyelephantopin, isodeoxy elephantopin, elephantol methacrylate, lupeol and so on. Research has assessed the antitumor activities of the sesquiterpene lactones from *E. scaber*[8], antibacterial activity of a new terpenoid from *E. scaber*[9], antidiarrhoeal and cardiotonic activity[10] and hypoglycemic effects of *E. scaber*[11].

MATERIALS AND METHODS

***Plant material*:** The leaves of *E. scaber* Linn. were collected during August-September of 2012, from the Campus of St.Thomas College, Palai, India. The plant was authenticated by Dept. of Botany, St. Thomas College, Palai, where a voucher specimen has been deposited.

General experimental procedures: IR spectrum was recorded on Shimadzu 8201 PC FT IR, JAPAN spectrophotometer. The ^{1}H NMR spectrum was recorded on a Bruker spectrometer (500 MHz) instrument using $CDCl_3$ as an internal reference. The chemical shift values were reported in ppm (δ) units. The ^{13}C NMR spectrum was recorded at 400 MHz on the same instrument. EI-MS were recorded on a Jeol JMS- HX 110 spectrometer with data system. Column chromatography was carried out using silica gel of 60-120 mesh. Aluminium sheets precoated with silica gel 60 F254 [20×20 cm, 0.2 mm thick. E-Merck (Darmstadt, Germany)]; were supplied by local authorized dealer of E-Merck were used for TLC to check the purity of the compounds isolated.

Extraction and isolation: The leaves of *E. scaber* Linn. were collected and shade dried at room temperature and ground into fine powder in an electric blender and subsequently sieved for obtained fine powder. 50 gms of the sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus using hexane, chloroform, acetone, methanol and water successively. Before extraction with the next solvent the powder was air dried to remove the adhering solvent. The extract obtained was filtered and concentrated in rotary evaporator. The concentrated plant extract of acetone was used for further antimicrobial assays and isolation of compounds. The acetone fraction was subjected to silica gel column chromatography using Toluene:Ethyl Acetate and Toluene: Ethyl Acetate: Acetone in an increasing polarity order to get 27 fractions (50ml each) were collected, of which 18 fraction showed antibacterial

activity. Fraction 18 was analyzed by TLC method to ascertain the purification with Toluene:Ethyl Acetate (9:1) yield the compound A (15mg).

8- hydroxyl naringenin: A yellowish liquid; IR v_{max} (KBr): 3443.05, 2928.04, 1730.21, 1462.09, 1274.99, 1120.68, 1078.32 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR spectral data are shown in Table 1; HR-EI-MS: m/z 291.086315 $[M+H]^+$.

Antibacterial assay (Disc diffusion method): The 12 standard bacterial strains used for the study were obtained from the Institute of Microbial Technology (IMTECH) Chandigarh, India. The isolated fraction were tested against 3 gram positive bacteria like *Bacillus cereus*, *Staphylococcus aureus* and *Streptococcus heamolyticus* and 9 gram negative bacteria like *Salmonella typhi*, *Entrobactor aerogenes*, *Vibrio cholera*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Serratia marcesens*, *Proteus rettigiri* and *Pseudomonas aeruginosa*. Various methods have been used to evaluate the antibacterial activity from time to time. The crude extract and isolated compound fraction 18 were screened for their antibacterial activity in comparison with standard antibiotic Amphicilin (6 mg/ml), in vitro by disc diffusion method using various bacterial strains[12]. The acetone crude extract and isolated fraction (10 µL) were loaded onto each Whatman filter paper disks (6mm diameter) and placed aseptically on the agar surface with the help of a sterile forceps and paper discs were pressed slightly with the forceps to make complete contact with the surface of the medium[13]. The plates were kept at room temperature for half an hour and subsequently incubated at 37°C and observed for zone of inhibition after 24 hours. The inhibition zone around each disc was measured in millimeter and the assay was carried out three times for each sample. The results were recorded by measuring the zone of growth inhibition surrounding the disc.

Culture media and inoculums preparation: Nutrient agar /broth (Himedia, India.) were used as the bacterial culture medium in the Bacterial assays. Loops full of all the bacterial cultures were inoculated in the 50 ml of sterile nutrient agar (NA) in 100 ml conical flask at 37 ºC for 72 hrs.

RESULTS AND DISCUSSIONS

A novel flavanone, compound A was identified by comparison of its physical and spectral data with literature values as 8-hydroxy naringenin[14-15]. Compound A was obtained as a yellowish liquid and the EI-MS displayed the molecular ion at m/z 291, whereas HR-ESI-MS analysis of the same ion gave the molecular formula $C_{15}H_{14}O_6$ (m/z 291.086315) with 7

double bond equivalents (DBE). The IR spectrum showed absorption bands at 3443.05 cm^{-1} and 2959.9 cm^{-1} indicating the presence of hydroxy group and 2928.04 cm^{-1} and 2864.39 cm^{-1} for aromatic and aliphatic –C-H stretching vibrations. The signals at 1730.21 cm^{-1} suggests the C=O stretching vibration of aromatic ring. An absorption at 1462.01 cm^{-1} indicates –C-H bending. The signal at 1274.99 cm^{-1} suggests the –C-C stretching vibration of aromatic ring. An absorption at 1120.68 cm^{-1} is due to the presence of –C-O group. IR spectrum also contained bands due to –C-O group of alcohol bending vibration at 1078.32 cm^{-1}.

The ^{1}H NMR and ^{13}C NMR spectral data were showed in Table 1. The ^{1}H NMR spectrum shows signals at δ7.888 (1H, 12, C-H proton), δ7.362 (1H, 14, aromatic proton), δ6.821 and δ6.721 (2H, 11,15, aromatic proton), δ3.448 (1H,4,OH proton), δ2.968 (1H, 2, OH proton), δ2.468 (1H, 5, OH proton), δ1.965 (1H, 1, aromatic proton), δ1.448 (1H, 3, aromatic proton), δ1.276 (1H, 6, aromatic proton) and δ1.268 (1H, 8, aromatic proton). In the ^{13}C NMR spectrum of the compound A, showed signals at δ174.428, δ174.366, δ162.836 and δ161.222 are consistent with the aromatic (benzene) ring carbons (C10, C11, C12, C13, C14 and C15). The aliphatic carbons showed signals at δ50.426, δ50.311 and δ50.267 (C5, C6, C8 and C9). The signals at δ106.256 and δ106.253 are due to the presence of alkene C-OH (C1, C2, C3 and C4) and alcoholic –OH (C2, C4 and C5) linkage. The elemental analysis was conducted by elemental analyzer for the compound A. The elemental analysis provided the following results; C (62.07%), H (4.86%) and oxygen (33.07%) and this lead to the molecular formula $C_{15}H_{14}O_6$. All the spectral data of compound A are revealed that the molecular formula $C_{15}H_{14}O_6$ and is 8-hydroxy naringenin and the structure of the compound is given below (Figure 1). The EI-MS of the compound A showed molecular ion peaks m/z 291; corresponding to the molecular formula $C_{15}H_{14}O_6$. This compound appears to be novel to the plant *E. scaber* Linn.

The crude acetone extract of *E. scaber* showed no significant antibacterial activity against the tested bacteria. But the isolated compound A from the acetone extract showed significant activity against 12 tested bacteria. The results of the antibacterial screening of acetone extract and isolated compound A are presented in the Table 2. The results revealed the inhibitory action of compound A against bacteria. Among the various gram positive bacteria, compound A showed maximum activity (zone of inhibition, 38.3 mm) against *Staphylococcus aureus* (Figure 2A), whereas moderate activity against *Streoptococcus heamolyticus* (zone of inhibition, 33.4 mm). While, the acetone extract of *E. scaber* showed no significant activity

against the tested bacteria. Similarly, among the gram negative bacteria tested, the compound A exhibited maximum activity (zone of inhibition, 40.5 mm) against *Salmonella typhi* ((Figure 2B). Whereas, the compound A showed moderate activity (zone of inhibition, 26.7 mm) against *Klebsiella pneumonia*. The inhibition of bacterial growth was dependent on the inhibitory activity of the sample. In this work, the isolated compound A from acetone extract of *E. scaber* Linn. showed more inhibitory activity compared to acetone extract.

The interaction of multiple compounds in the crude extract reduced the antibacterial activity of acetone extract. In the case of isolated compound A, 8- hydroxyl naringenin showed maximum activity because there was no synergetic effect. Flavonoids are a diverse group of polyphenolic compounds widely distributed in several plant species with a variety of biological activities. In an earlier report the compound 8-hydroxynaringenin has been reported as a suicide substrate of mushroom tyrosinase[15]. The compound 8-hydroxynaringenin isolated from *E. scaber* demonstrated broad spectrum of antimicrobial activities and therefore, these findings provided rationale for the wide range of medicinal applications especially ethnomedicinal utilization of this plant in traditional medicine.

Table 1: ^{1}H (500 MHz) and ^{13}C (400 MHz) NMR spectral data (δ values in $CDCl_3$)

Position	^{1}H	^{13}C
1	1.965	70.264
2	2.968	70.198
3	1.448	70.192
4	2.863	68.382
5	2.468	-
6	1.276	50.426
7	-	50.311
8	1.268	50.267
9	-	-
10	-	-
11	6.821	174.428
12	7.888	174.368
13	3.448	106.256
14	7.362	162.836
15	6.721	161.222

Table 2: Antibacterial activity of crude acetone extract and isolated compound A of *E. scaber*

Bacteria	Zone of Inhibition (mm)	
	Acetone Extract	Compound A
Bacillus cereus	7.3	35
Staphylococcus aureus	7.1	38.3
Streptococcus heamolyticus	6.9	33.4
Salmonella typhi	-	40.5
Entrobactor aerogenes	-	28.2
Vibrio cholera	-	29.4
Escherichia coli	6.5	32.6
Proteus vulgaris	-	30.2
Klebsiella pneumonia	-	26.7
Serratia marcesens	-	27.5
Proteus rettigiri	-	26.9
Pseudomonas aeroginosa	-	29.8

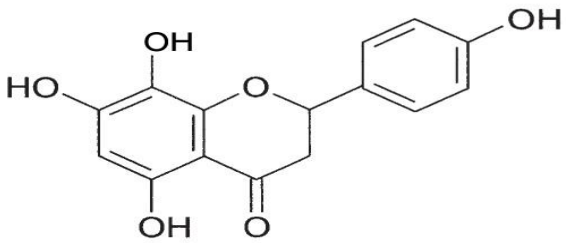

Figure 1: Structure of compound A, 8- hydroxyl naringenin

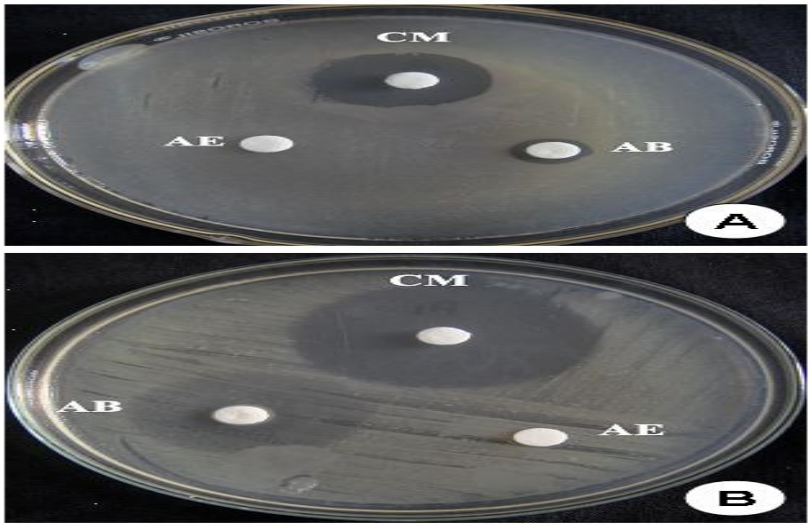

Figure: (2A): Antibacterial activity of acetone extract and 8-hydroxy naringenin from *E.scaber* against *Staphylococcus aureus.* CM= Compound, AE= Acetone extract, AB= Antibiotic disc. (2B): Antibacterial activity of acetone extract and 8-hydroxy naringenin from *E.scaber* against *Salmonella typhi.* CM= Compound, AE= Acetone extract, AB= Antibiotic disc

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