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A New Aromatic Compound from Edible Flavouring Herb and Its Bioactivity

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Tetrahydro-5-(hydroxymethyl)-6-(1,2-diphenylvinyl)-2*H*-pyran-2,3,4-triol having CNS depressant property was isolated by column chromatography from the leaves of an edible flavouring herb and was identified by IR, ^{1}H, ^{13}C NMR and LC-MS.

Keywords: *Eryngium foetidum*, Aromatic, Hypnotic, CNS depressant.

INTRODUCTION

Wild edible products are obtained not only from different parts of numerous plants such as roots and tubers, stems, leaves, flowers, fruits *etc*., but in some cases the entire plants too is edible. People of Tripura, India, use leaves of *Eryngium foetidum* (F: Umbelliferae) as vegetables and for flavouring curries. This plant is a diffuse herb with fusiform root, leaves simple, spathulate and leaf margin spinous toothed, flower white in colour with pubescent spinous. Edible part *i.e*., leaves of *E. foetidum* is aromatic[1].

E. foetidum L. is a biennial herb indigenous to continental tropical America and West Indies. The herb is used extensively in the Caribbean, Latin America, the Far East and in Asia particularly in India and Korea. This plant is widely used as herbal medicine and reportedly beneficial in the treatment of a number of ailments[2]. Drugs from plant origin are used in India for treatment of many diseases in traditional system of medicine[3]. This plant has the traditional uses in fever and chills, vomiting, convulsions, pneumonia, flu, diabetes, malaria fever, ear ache, analgesic, antiinflammatory, hypertension, fits, asthma, stomachache, infertility complications, snake bites and an appetite stimulant, *etc*.[4-7]. *E. foetidum* has no clastogenicity, but possesses anticlastogenic potential against both direct- and indirect-acting types of clastogen in mice[8]. Topical antiinflammatory activity of *E. foetidum* in chronic and acute model was reported by Garcia *et al*[9]. It is also reported that *E. foetidum* leaf extract possesses suppressive effects against pro-inflammatory mediators and so it has a high potential to be used as a food supplement to reduce risk of cancer, associated with inflammation[10]. Significant hyperglycaemic activity of *E. foetidum* leaves were reported by Chandira *et al*[11]. The LD_{50} value for *Eryngium foetidum* leaves extract (1649.24 mg/Kg) and the analgesic-antiinflammatory activities were also reported[12].

Chemical evaluation of the leaves indicated the presence of flavonoids, tannins, saponins and triterpenoids; but no alkaloids were reported[6]. A significant constituent of the essential oil of the plant is E-2-dodecenal ("eryngial"). Eryngial showed its effectiveness against parasitic trypanosomes, nematodes, fungi and bacteria in humans and other mammals[6-13]. Some major compounds were also identified in essential oil of leaves of *Eryngium foetidum* and those are dodecanoic acid, *trans*-2-dodecanoic acid, (E)-2-tridecenal, duraldehyde and tetradecanal[14]. In addition, Wang *et al.*[15] reported that nonessential oil obtained from *E. foetidum* also contains coumarins, polyacetylenes and steroids.

In continuation of our search on herbal medicines[16-18], the present compilation is reporting a new aromatic compound [tetrahydro-5-(hydroxymethyl)-6-(1,2-diphenylvinyl)-2*H*-pyran-2,3,4-triol] isolated from the leaves of *E. foetidum* having CNS depressant property.

EXPERIMENTAL

The leaves of *Eryngium foetidum* were collected from Agartala, Tripura, in the month of September' 2011 and were shed dried. The dried leaves were then crushed to fine powder content and dipped in methanol (100 g/100 mL) for 3 days and filtered. Filtrate was subjected to further use.

Separation: Components present in the extract were separated by following column chromatography technique taking ethyl acetate as mobile phase and silica gel as stationary phase.

The column used was 50 cm in length and 2 cm in diameter. Separated fractions were collected in individual beakers. Total 6 fractions were obtained (fraction 1- 60 mL, fraction 2- 50 mL, fraction 3- 65 mL, fraction 4-110 mL, fraction 5-90 mL, fraction 6- 85 mL, absorbed by silica gel-40 mL). The liquid portion of largest volume (fraction 4) among the fractions collected was then allowed to evaporate till dry (yield - 0.07 g) and this was used for structure elucidation, as LC-chromatogram of this compound showed single peak. Further, studies of hypnotics as well as CNS depressant activity of this compound were also carried out.

Spectral analysis: The IR spectra were recorded in JASCO FTIR-5300 in KBR pellets. ^{1}H, ^{13}C NMR were recorded by using DMSO-d_6 + $CDCl_3$ Mix in Bruker AC-F 300 FTNMR spectrophotometer. Mass spectra were obtained with LC-MS Shimadzu 2010A.

^{1}H NMR spectrum exhibits peak at δ 3.03, 4.55, 4.53, 4.51, 4.48, 7.52 ppm. ^{13}C NMR spectrum shows peaks in the region d 78.32, 78.96, 79.63, 134.51, 121.56, 127.41, 129.93, 128.51, 124.96, 128.11, 126.93, 133.60, 124.44, 125.38, 129.63, 129.26 and 125.17 ppm. Mass spectra is recorded at *m/z* 365, 266, 180, 164, 104.

Bioactivity: Hypnotic activity[19] was carried out by studying the effect on righting reflex in mice. The study for CNS depressant activity[19] was carried out by studying the locomotor activity of mice using an actophotometer. For pharmacological evaluation adult Albino swiss mice of either sex (20-25 g) have been used. The experimental data obtained for the test compound were compared with the data of standard such as pentobarbitone at a dose of 50 mg/Kg bw for hypnotic activity and chlorpromazine HCl at a dose of 5 mg/Kg bw for CNS depressant property. The intraperitoneal dose of test compound was fixed as 100 mg/Kg bw. Control group was also taken for each case and treated with the vehicle *i.e*., normal saline. Each group was containing 10 mice. The animals were housed under standard environmental condition (25 ± 2 °C) and relative humidity (50 ± 5 %) and fed with standard diet and water *ad libitum*. The animals were acclimatized to laboratory environment for a period of 4 days before performing the experiments.

To observe the hypnotic activity, pentobarbitone was administered and then after 5 min interval the test compound was also injected. The onset of action on mice was recorded by noting the time when the animal lost its righting reflex. The time of recovery from sleep was also noted down by recording the time when the animal recovers its normal posture. The average onset and duration of action were calculated ($P < 0.001$). For CNS depressant activity of the compound, individually each mouse of each group was kept in an INCO-photoactometer for 5 min before treating with drugs. The drugs were administered later and then retested individually. The average per cent decrease of movement (scores) *i.e*., locomotor activity values were calculated ($P < 0.001$), which indicates CNS depressant property.

RESULTS AND DISCUSSION

A new aromatic compound was isolated from the leaves of *E. foetidum* by using column chromatography. IR spectrum of the purified compound exhibits a band at 3531 cm^{-1} for the presence of OH group and also at 3620 cm^{-1}, 1155 for C-O str of tetrahydropyran and 1462 cm^{-1} for aromatic C=C skeletal vibration. In its ^{1}H NMR spectrum, it exhibits intense peak at δ 3.03 ppm and peak at δ (4.55, 4.53, 4.51, 4.48) ppm for the hydroxyl group and protons under hydroxyl function indicating the presence of a sugar like moiety. Apart from these peaks, there is only one peak at δ 7.52 ppm indicating the presence of aromatic ring protons. ^{13}C NMR spectrum shows peaks for sugar like moiety in the region δ 78.32, 78.96, 79.63 ppm. The aromatic peaks as appeared in ^{13}C NMR spectrum are described as per the predicted structure drawn (Fig. 3): For carbon 1" *i.e*. C1" at δ 134.51 ppm, C2" at 121.56, C3" at 127.41, C4" at 129.93, C5" at 128.51, C6" at 124.96, C7 at 128.11, C8 at 126.93, C1' at 133.60, C2' at 124.44, C3' at 125.38, C4' at 129.63, C5' at 129.26 and C6' at δ 125.17 ppm. LC chromatogram shows the single peak of mAbs at 0.568 min in 254 nm, which proofs the purity of the compound. The molecular mass of the compound is deduced from its LC-MS ESI positive mode spectrum by the presence of the pseudo molecular ion peak at *m/z* 365 for $[M + Na]^+$ ion. The compound after loosing the sugar moiety exhibits an ionic peak at *m/z* 180 (Fig. 1) and further it looses one benzene ring to an ion at *m/z* 104 (base peak: Fig. 2). With the above facts a characteristic structure is deduced for the purified compound isolated from the leaves of *E. foetidum* (Fig. 3 tetrahydro-5-(hydroxymethyl)-6-(1,2-diphenylvinyl)-2*H*-pyran-2,3,4-triol).

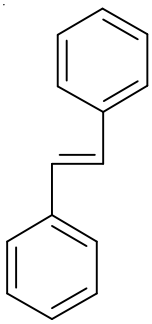

Fig. 1.

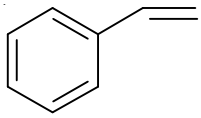

Fig. 2.

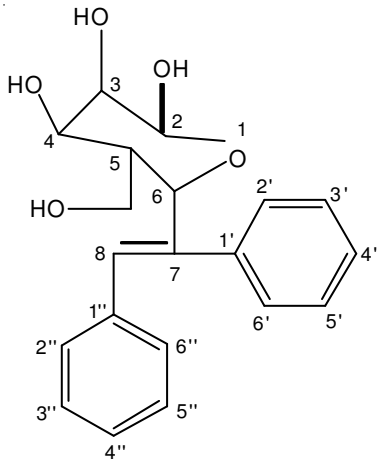

Fig. 3.

Average of the total duration of sleep for the study of hypnotic activity was recorded 38.7 min (+SEM: 0.843) for the compound obtained from *E. foetidum*, 42.2 min (+SEM:

0.571) for pentobarbitone and 42 min (+SEM: 0.343) for control. Again the mean time for the onset of action (in min) after pentobarbitone injection was 6.9 (+SEM: 0.734), while it was 11.3 (+SEM: 0.745) for the compound and 7.1 (+SEM: 0.451) for control. These results indicate that the compound was neither supporting the onset of action nor sleeping time, induced by pentobarbitone. Hence the test compound did not show hypnotic activity. The study of CNS depressant property, found by percentage change (mean) of decreasing activity *i.e.* decreasing of movement (scores), recorded 52.83 (+SEM: 0.876) in case of compound and 58.02 (+SEM : 0.974) in case of chlorpromazine, whereas for control the decrease of scores was not observed. This observation suggests that the compound tetrahydro-5-(hydroxymethyl)-6-(1,2-diphenylvinyl)-2*H*-pyran-2,3,4-triol obtained from *E. foetidum* leaves though has CNS depressant property, but not at par to the standard.

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