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GC-MS analysis of terpenoids from *Eranthemum roseum* (Vahl) R. Br.

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ABSTRACT

This works describes the simplest method for the identification of terpenoids and steroids from roots of *Eranthemum roseum* (Vahl) R.Br. (Acanthaceae), a medicinal plant widely used in India. By using gas chromatography coupled to mass spectrometry (GC-MS), six terpenoids and steroids were identified, all of them reported for the first time in *Eranthemum roseum.*

Keywords: Thandikarav, Gas Chromatography- Mass spectrometry (GC-MS), *Eranthemum roseum.*

INTRODUCTION

The Acanthaceae family comprises approximately 234-259 genera and about 2599 species. Most are tropical herbs, shrubs or twining vines; some are spiny. Only a few species are distributed in temperate regions. Various species of genus Eranthemum are being used traditionally for wide verities of ethno medicinal purposes. The *Eranthemum roseum* commonly known as Gulsham or Dasmuli 1. Tribals of Nandurbar district call " Thandikarv" . Ethno botanically roots of *Eranthemum roseum* boiled with milk is a popular remedy for Leucorrhoea 1. Roots are also given to pregnant cattle of promote the foetus growth.2. In folkure medicine, it is natural remedy for ulcer (Personal communication with aadivasi people of Nandurbar district). Its roots are used as antimicrobial activities were tested against different bacteria and fungi 3. It is also useful for Pharmacognostical study and Physico-chemical standardization of herbal drugs4. Roots are reported to Gastroprotective effects of Albino Rats.5 Despite the popular use of this species as a medicinal plant, there is no scientific data available regarding its phytochemistry and pharmacological effect.

MATERIAL AND METHODS

Plant Material: Roots of *Eranthemum roseum* were collected from Satpuda valley, Dist. Nandurbar, Maharashtra, India. The plant was authenticated at Department of Botany, P.S.G.V.P.M's College of Science, Shahada , Maharashtra. The voucher specimen has been kept at Department of Botany, Science College, Shahada. The roots were shed dried, ground and sieved with a 40 mesh sieve.

Extraction of Plant Material: 100gm roots powder of *Eranthemum roseum* were extracted with petroleum ether. After filtration, the acidic compounds were extracted out with 5 % aqueous KOH (three times) followed by the extraction of the basic compounds with 5 % aqueous HCL (three times). The organic fraction, which contain the neutral compounds, was washed with water to pH -7 and concentrated in rotary vacuum evaporator to 50 ml. Suspended particle were removed by centrifuging the concentrated extract for 10 min at 6000 rpm. Then solvent was evaporated to dryness, giving a residue, which was dissolved in chloroform for GC-MS analysis. Fig. 1 summarizes the process of extraction.

Chromatographic analysis: GC-MS analysis was performed on a Hewlett-Packard 5890 gas chromatograph, with a split injector (1:50) at 280° C and a Hewlett-Packard 5970 mass selective detector (MSD), with the GC-MS interface temperature at 280 ºC. The injection volume was 2µl. Hydrogen was employed as carrier gas, at a pressure of 60 kPa. A HP-1 25m x 0.25mm x 0.33 µm methylpolysiloxane cross-linked capillary column was employed with temperature programming from 100 ºC (held for 2 min) to 280 ºC (held for 30 min) at a ratio of 4 ºC/min.

RESULT AND DISCUSSION

Figure 2 shows the total ion chromatograms (TIC) of Petroleum ether extract of *Eranthemum roseum* roots. For the characterization of the compounds detected in TIC/GC-MS, a process which increased the confidence of the identification of compounds by the mass spectrometry was applied. Firstly, the fragmentogram obtained for each compound was compared with the fragmentation data base of the mass spectrometer, for obtaining a list of the 10 most probable substances (tentative identification). Sweeping was done by using the SCAN mode, in which the values of m/z between 50 and 480 Daltons were chosen. It was observed that, at the beginning of each probable fragmentogram, that the fragments of the compounds with tR 40 to 56 min had the same fragmentation between 50 and 120 Daltons, and that differences appeared only after 120 Daltons.

The sample were analyzed again using the SCAN mode, but with the sweeping range set between m/z 120 and 550. In this new sweeping , several fragments that showed lower intensity (5 to 10 % abundance) and have not been useful for comparison with standards library data, turned to 100% abundance, therefore participating in the comparison with NBS library (around 40,000 compounds). This provided alternatives for the more probable compounds, with a higher "match quality" score for the analyzed compounds.

The result of GC-MS analysis of *Eranthemum roseum* were given in Table no. 1, shows GC-MS experimental data, retention time (RT), and mass fragment of compounds for terpenoids and steroids of *Eranthemum roseum* .Individual compound were identified from RT, mass data and by comparison of the data of standard compounds with those of in the literature. Six compounds viz. Lupeol, Campesterol, Stigmasterol, Beta- sitosterol acetate, Squalene, Phytol were identi-

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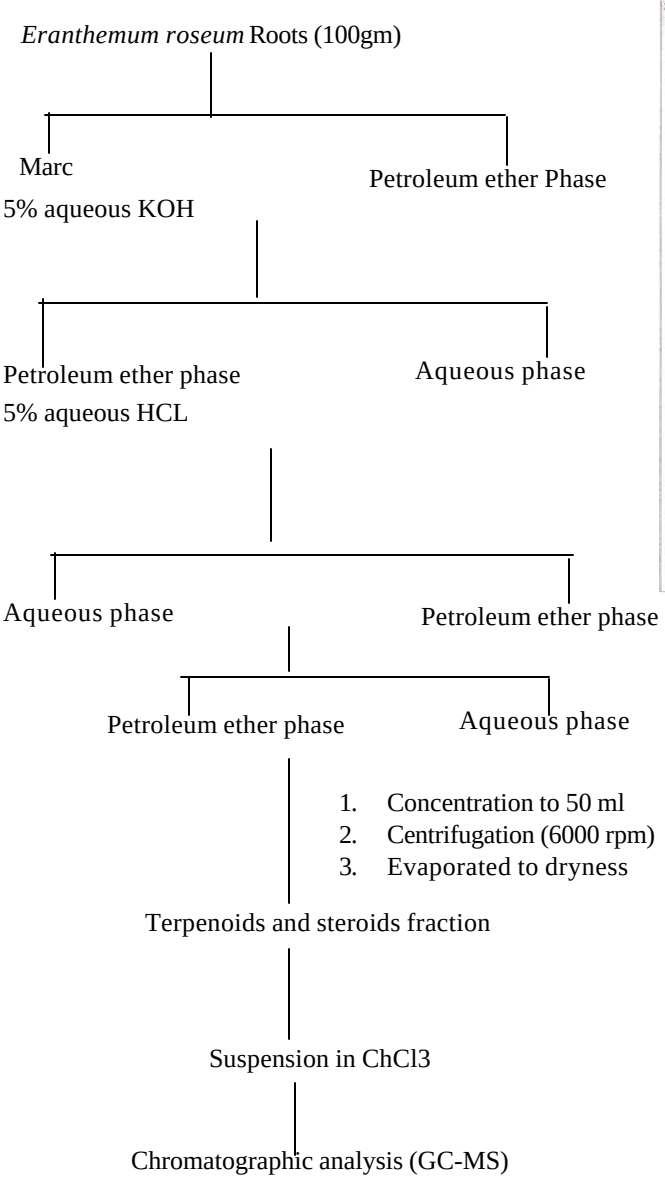

Figure 1. Extraction procedure of terpenoids and steroids of *Eranthemum roseum*

Table No. 1 The mass fragment of identified components from *Eranthemum roseum* by GC-MS.

Compound	Retention Time	M+	Main fragments	%[a]
Lupeol	42.62	426	424,409,368,355,313,245,205,121,109	1.41
Campesterol	40.05	400	397,396,381,329,275,213,159,145,105	3
Stigmasterol	37.65	412	395,394,379,351,255,213,159,145	3.65
Beta-Sitosterol	36.79	456	439,400,382,367,261,255,213	4.77
Squalene	26.58	410	367,257,231,177,161,135,121,81,69	3.79
Phytol	19.78	296	278,193,123,109,97,81,71,57,43	2.13

a- The area of GC-MS peak depend not only on concentration of corresponding compound, but also on the intensity of their mass spectral fragmentation, so the data given in table is not true quantitation but can be used for comparison between two samples.

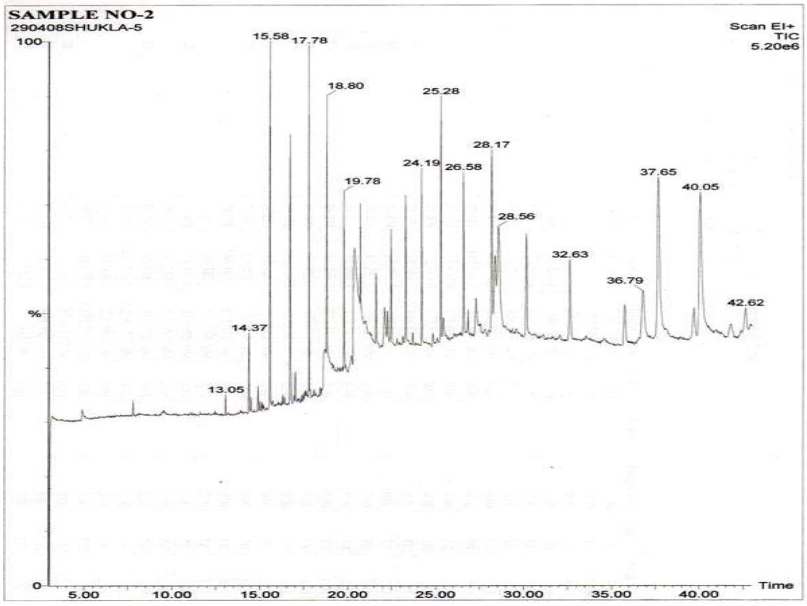

Figure 2 The total ion chromatograms (TIC-GC-MS) of Petroleum ether extract of *E .roseum* roots (Peaks: 1- Lupeol (42.62), 2- Campesterol (40.05), 3- Stigmasterol(37.65), 4- Beta-sitosterol (36.79), 5- Squalene (26.58), 6- Phytol (19.78))

fied in *Eranthemum roseum.*

CONCLUSION

Therefore, GC-MS method is direct and fast analytical approach for identification of terpenoids and steroids and only few grams of plant material is required. The importance of the study of terpenoids is due to the biological activity of some of these compounds. The present study, which reveals the presence o terpenoids and steroids in *Eranthemum roseum* suggest that the contribution of these compounds on the pharmacological activity should be evaluated.

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