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RESEARCH ARTICLE

Gas Chromatography-Mass Spectroscopy Analysis of *N*-Hexane Extract of *Epaltes pygmaea* DC

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ABSTRACT

The present study was aimed at finding the chemical composition of n-hexane extract of whole plant of *Epaltes pygmaea* DC. (Asteraceae) by gas chromatography-mass spectroscopy (GC-MS). Fifteen different compounds were characterized by GC-MS. This is the first attempt to investigate the GC-MS analysis of *Epaltes pygmaea* DC. The major constituents are *n*-hexadecanoic acid, (Rt 21.80), ethyl hexadecanoate (Rt 22.4), ethyl (9Z, 12Z)- 9,12-octadecadienoate (Rt 25.08), octadecanoic acid ethyl ester (Rt 25.55), hexacosane (Rt 31.06). These constituents were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. The result of present studies enhance that the plant *E. pygmaea* possess several known and unknown bioactive compounds.

KEYWORDS

GC-MS Analysis, *Epaltes pygmaea*, *N*-Hexane Extract

INTRODUCTION

The importance of medicinal plants has been overlooked in the past. However, at present, medicinal plants are looked upon not only as a source of cost effective affordable health care but also as a source of income to cultivators. According to the WHO, about 80% of the population in developing countries relies on traditional medicines for their primary health care. Most of the traditional medicines are plant-based drugs. Moreover 20% of prescription of drugs is presently derived from plants[1]. In traditional medicine, the medicinal plants still play a vital role in catering to the basic health needs, since the use of herbal remedies has increased tremendously in the developed countries in the last decade[2].

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India has over one million traditional village level healers and several million of knowledgeable households, who are well versed in traditional home remedies. Herbal remedies have attained much popularity in the treatment of minor ailments due to the increasing awareness of personal health maintenance through natural products[3]. In this connection, plants continue to be a rich source of therapeutic agents. The remarkable contribution of plants to the drug industry was possible because of the large number of phytochemical and biological studies all over the world.

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical on ethno-pharmcological information is generally

considered as an effective research based approach in the discovery of new anti-infective agents from higher plants[4]. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies[5]. Chemical constituents may be therapeutically active or inactive. The ones which are active are called active constituents and the inactive ones are called inert chemical constituents[6].

Inspite of the enormous herbal potentials in India, only a fraction of the herbal wealth has come to limelight. There are thousands of new and little known plants, which deserve comprehensive scientific standardization and evaluation. In developing countries, ailments such as jaundice, urethral discharges, acute dyspepsia still prevail in noticeable extents and potential drugs used in those treatments possess diuretic, diaphoretic and stimulating expectorant natures. Plants medicines possessing these, although many, still lacks the complete exposure and research due to the lack of proper investigation on many such plants.

One among the uncovered medicinal weed *Epaltes pygmaea* belonging to the family Asteraceae and the genus *Epaltes*. Asteraceae (Compositae) is an advanced and botanically highly specialized family of mainly herbaceous plants. Large number of species in Asteraceae contains a wide variety of chemical constituents and literature has accumulated enormously. Latex and inulin are the two chemical active principles of Asteraceae. Rest of the compounds reported is volatile oils, sesquiterpenes, alkaloids, flavonoids and saponins. The genus *Epaltes* are used in traditional Ayurvedic medicine in Sri Lanka used to cure various ailments like jaundice, urethral discharges and acute dyspepsia, diuretic and stimulating expectorant[7].

Epaltes pygmaea DC. is a small annual herb, 8 to 20 cm high with minutely winged branched stem and aromatic roots. It is region specific and found mostly in Sri Lanka, also available in South India in minor quantities especially towards the coast, gregarious in low lying ground by river banks and paddy field after harvesting in clayey soil[8-13]. Some of the chemical constituent's lupeol acetate, stigmasterol, stigmasterol acetate, apigenin, luteolin, apigenin-7-0-glucoside and luteolin-7-0-glucoside were reported previously[14]. The whole plant of *E. pygmaea* has been reported for its hepatoprotective and anti-diuretic activities. Phytochemical screening studies have been carried out and reported the presence of flavonoid, coumarin, steroid, phenol, tannin, sugar, triterpenoids and amino acids[15]. Therefore, the present study was aimed to explore the remaining phytochemical constituents of *Epaltes pygmaea* by GC-MS analysis.

MATERIAL AND METHODS

Collection of Plant material

Plant material was collected during the flowering season in the month of February from Thirunelveli Dist of Tamilnadu and the identity of the plant was confirmed by Dr. V. Chelladurai Research Officer, (Retd) Botany, SRMU unit, Palayamkottai (C.C.R.A.S. Govt of India). The voucher specimen (00630) was deposited in Captain Srinivasa Murti Drug Research Institute for Ayurveda, Chennai. Plants were washed thoroughly running tap water to remove soil particles and adhered debris. Plants were cut, shade dried, ground in to fine powder and stored in air tight containers until use.

Preparation of Plant Extract

Shade dried and powdered plant material (4 g) was extracted with *n*-hexane using Soxhlet apparatus. The extract was filtered through Whatmann No. 1 filter paper to obtain particle free extract and then the extract was pooled, concentrated and dried under using vacuum pressure. This extract was subjected to GC-MS investigation.

Instrument

Shimadzu GC-MS QP2010 was the instrument used for gas chromatography-mass spectroscopy (GC-MS) analysis. The constituents were identified comparing the data with the existing software libraries like WILEY08, NIST08, NIST08s.

GC MS Analysis

One microliter of the extract of the plant was injected into the Gas chromatography unit. The injector temperature was maintained at 250ºC. The detector used was flame ionization detector which was maintained at 280ºC. The pressure of the carrier gas, nitrogen, was kept at 10 psi. The oven temperature was set at 60ºC to 280ºC with a gradual increment of 10ºC per min. The injected extract was separated into various constituents with different retention time which are detected extract was eluted in the DB-5 MS column of 30 m long and 0.25 mm inner diameter and the eluted constituents were detected by flame ionization detector and the GC chromatogram was recorded by mass spectrophotometer. The chromatogram a plot of intensity against retention time was recorded by the software attached to it.

RESULTS AND DISCUSSION

The *n*-hexane extract of the *E. pygmaea* was analysis by using GC-MS had led to the identification of twenty one different components. The active principles with their retention time (RT) and concentration (peak area%) are presented in Table-1. The GC-MS chromatogram of the twenty one peaks of the compounds detected was shown in Figure-1 in which 15 compounds were identified comparing the data with software libraries, from the result the major components are *n*-hexadecanoic acid, (Rt 21.80), ethyl hexadecanoate (Rt 22.4), ethyl (9Z, 12Z)- 9,12-octadecadienoate (Rt 25.08), octadecanoic acid ethyl ester (Rt 25.55), hexacosane (Rt 31.06) were found to be 9.39, 16.45, 26.46, 5.36, 7.25% respectively. The percentage area of the other minor components were < 4%.

Previous studies determined the chemicals constituents of *E. pygmaea* presence of flavonoids (luteolin, luteolin-7-O-glucoside, apigenin, apigenin-7-O-glucoside), steroids (stigmasterol) and triterpenoids (lupeol acetate). A number of scientific reports indicated that luteolin, luteolin-7-O-glucoside and apigenin, apigenin-7-O-glucoside *n*-hexadecanoic acid have the property of antibacterial, antioxidant[16], diuretic and hepatoprotective activity[17,18]. *n*-hexadecanoic acid also have larvicidal effect[19]

Therefore, GC-MS method is a 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 is due to the biological activity of some of these compounds.

CONCLUSION

In the present study fifteen chemical constituents have been identified from *n*-hexane extract of the whole plant of *E. pygmaea* by Gas Chromatogram- Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of the whole plant for various ailments. It could be concluded that *E. pygmaea* contains various bioactive compounds; it contributes to the medicinal activity of the plant. Further studies are required for support the therapeutic claims.

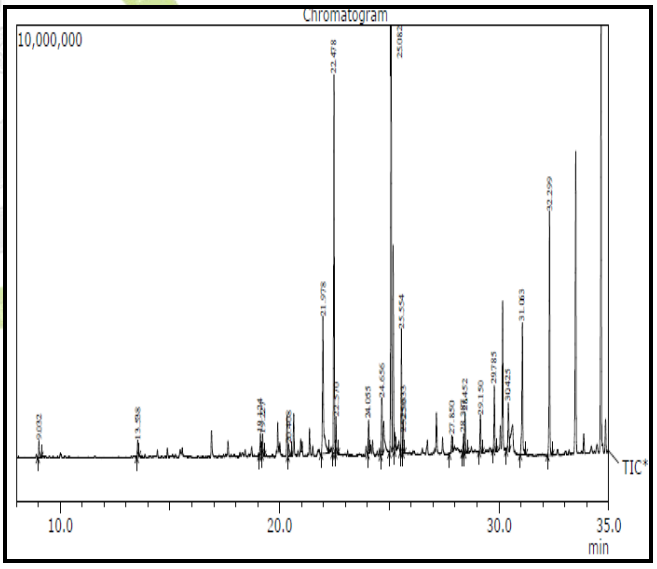

Figure 1: GC-MS chromatogram of hexane extract of *E. pygmaea*

Table 1: Phytoconstituents present in hexane extract of *E. pygmaea* using GC-MS

Retention time (min.)	Name of the compound	Area %
19.125	Tetradecanoic acid, ethyl ester	0.98

19.225	Octadecane	0.83
21.864	*n*-Hexadecaoonoic acid	9.39
22.475	Ethyl hexadecanoate	16.45
22.567	Eicosane	1.62
24.058	8,11-Octadecadienoic acid	1.48
24.658	9,12-Octadecadienoic acid (Z,Z)	3.20
25.083	Ethyl (9Z,12Z)- 9,12-Octadecadienoate	26.46
25.250	9-Octadecenoic acid (Z)-, ethyl ester	0.81
25.550	Octadecaonoic acid, ethyl ester	5.36
25.633	Docosane	1.38
28.383	Ethyl icosanoate	0.93
28.450	Tetracosane	1.83
30.425	1,2-Benzenedicarboxylic acid,mono (2-ethylhexyl) ester	2.62
31.067	Hexacosane	7.25

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