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Preliminary Phytochemical Screening and GC-MS Analysis of Methanolic Extract of *Turnera subulata* Smith (Passifloraceae)

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ABSTRACT

The investigation was carried out to determine the possible phytochemical constituents from aqueous, methanol and chloroform extracts of *Turnera subulata* leaf extracts. Among the phytochemical screening of these extracts, Methanolic extract showed that the leaf was rich in alkaloids, flavonoids, glycosides, phenols, saponins and quinones. The chemical composition of the plant leaf extract of *T. subulata* was investigated using Gas Chromatography – Mass Spectroscopy (Agilent-7890A GC instrument coupled with MS-5975) and NIST-MS library. GC-MS analysis of *T. subulata* plant leaf extract, revealed the existence of the GC-MS chromatogram of the major peaks presented in methanolic extract like *Methyl 8,11,14-heptadecatrienoate* (23.244%), *Pentadecanoic acid, 14-methyl-,methyl ester* (8.654%), *n-Hexadecanoic acid* (8.654%), *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl* (6.598%), *1b,4a-Epoxy-2H-cyclopenta[3,4] cyclopropa[8,9]cycloundec[1,2-b] oxiren-5(1aH)-one*(5.400%), *3,7,11,15-Tetramethyl-2-hexadecen-1-ol*(5.400%), *etc*. From this study it is obvious that *T. subulata* leaf extract contains many biologically active compounds and also it gives a detailed insight about the phytochemical profile which could be exploited for the development of plant based drug.

Keywords: *Turnera subulata*, GC-MS, Methanol, Methyl 8,11,14-heptadecatrienoate, Pentadecanoic acid, 14-methyl-,methyl ester.

INTRODUCTION

The flora and fauna of mother earth has a great diversity. The number of plant species divided in about 300 families and 10,500 genera are supposed to be about2-2.5 lacs. Atleast 100-150 species of medicinal plants are currently cultivated and about 30-40 of them are the large scale field crops. The export of medicinal plants and herbs from India has been quite substantial for the last few years. India has a large endemic flora. There are more than 80,000 medicinal plants known, and nearly 180 plant-derived chemical compounds have been developed as modern pharmaceuticals, which are included in the Pharmacopoeia of India. The WHO estimates that about 80% of the populations living in the developing countries rely exclusively on traditional medicine for their primary health care needs [1]. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency [2]. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [3,4].These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [5].

Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds [6]. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [7,8]. Therefore, proper scientific knowledge is required to investigate and explore the exact standardization of such medicinally important plant. Mass spectrophotometry coupled with chromatographic separations such as Gas chromatography (GC-MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants [9]. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [10].

The present investigation was carried out to determine the possible chemical components from the methanolic extracts of leaves in *Turnera subulata* by GC-MS.

MATERIALS AND METHODS

Collection of Plant Material

The flowering plants of *T. subulata* Smith was collected from location Tiruchirappalli, Tamilnadu situated in the southern region of India during the month of May 2017. The plant material was identified and authentication with taxonomically using, the Flora of Tamil Nadu Carnatic [11], the identified plant samples were then confirmed to the Department of Botany, Bishop Heber College, Tiruchirappalli (Figure 1).

Figure 1: *Turnera subulata* Smith.

Preparation of the Extract

The plant material (leaves) was soaked in Methanol (Leishman's stain) for 24 hrs. at room temperature, chopped into small pieces, ground into crude extracts and was placed into the extractor of a centrifuge. The extraction was carried out using methanol solvent. At the end of the extraction the solvent was concentrated by evaporation. The obtained extracts were stored in a refrigerator at 4^{o}C for further analysis.

Preliminary Phytochemical Screening

Phytochemical examinations were carried out for the leaf extracts of *T. subulata* as per the standard methods.

1. Detection of alkaloids

Mayer's test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of yellow colored precipitate indicates the presence of alkaloids [12].

2. Detection of carbohydrates

Benedict's test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars [13].

3. Detection of glycosides

Modified Borntrager's test: Extracts were treated with Ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides [12].

4. Detection of saponins

Foam test: 1ml of extracts was shaken with 2ml of water. If foam produced persists for ten minutes it indicates the presence of saponins[13].

5. Detection of phytosterols

a) Salkowski's test: Extract was treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes [14].

b) Libermann-Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled Con. Sulphuric acid was added. Formation of brown ring at junction indicates the presence of phytosterols[15].

6. Detection of phenols

Ferric chloride test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols [16].

7. Detection of Flavonoids

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour indicates the presence of flavonoids [17].

8. Detection of proteins and amino acids

Ninhydrin test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of proteins and amino acid [18].

9. Detection of diterpenes

Extracts were treated 2 ml of chloroform. Add 2 to 3 ml of sulfuric acid. Formation of brown colour ring at the junction indicates the presence of diterpenes.

10. Copper acetate test

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

GC- MS Analysis

GC-MS analysis of methanolic extract of *T. subulata* was performed in an Agilent-7890 A GC instrument coupled with MS-5975 inert MSD and triple axis mass selective ion detector. The DB-5MS column with dimensions of 30m x 0.2mm capillary column was used for the analysis. The initial temperature was kept at 150 °C and the maximum of 300 ° C. One 1µl of sample was injected with split mode

(10:1). Helium gas used as a carrier gas at flow rate of 0.8 ml/min and the total run time was 22 minutes. Identification of phytochemical components was conducted using the database of National Institute Standard and Technology MS library (NIST- MS library).

RESULTS

Phytochemical Analysis of the various leaf extracts of *Turnera subulata* Smith.

Phytochemical analysis of various leaf extracts of *Turnera subulata* was subjected to preliminary phytochemical screening and the phytoconstituents reports are given in Table 1. The maximum percentage yield was obtained in methanol extract followed by water and chloroform. Most of the secondary metabolites present in methanolic extract like Alkaloids, Carbohydrates, Glycosides, Saponins, Phytosterols and Flavonoids are reported.

Table 1: Phytochemical screening of the various extracts of *Turnera subulata* leaf:

S.No.	Phytochemical	Water	Methanol	Chloroform
1	Alkaloids	+	+	+
2	Carbohydrates	-	+	+
3	Glycosides	-	+	+
4	Saponins	+	-	-
5	Phytosterols:			
	▪ Salkowski's test	-	+	+
	▪ Libermann-Burchard's test	+	+	+
6	Phenols	-	+	+
7	Flavonoids	-	+	+
8	Proteins and Amino acid	-	+	+
9	Diterpenes	-	+	+

GC-MS Analysis of the methanolic leaf extracts of *Turnera subulata* Smith.

The bioactive compounds present in the methanolic extract of *T. subulata* leaves using Gas chromatography coupled with Mass spectroscopy (GC-MS) reports are given in Figure 2.

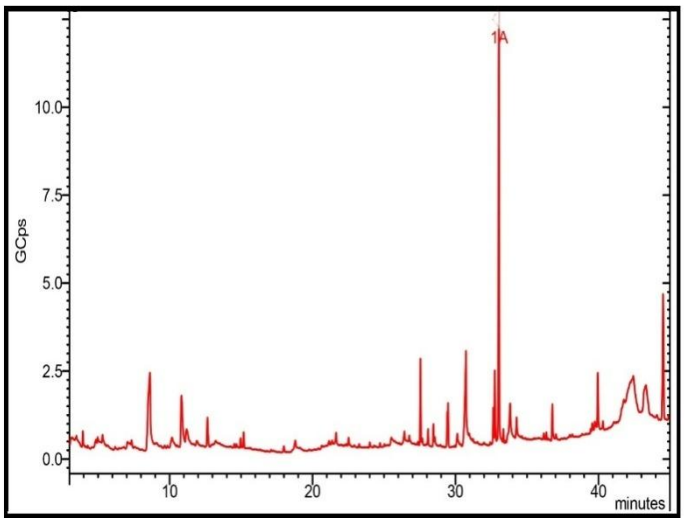

Figure 2: Chromatogram of Methanolic Extract of *T. subulata* Smith

In the methanolic sample, 22 compounds were identified and the highest percentage content of peak area of 23.244 (Methyl 8,11,14-heptadecatrienoate with Rt 32.751min), followed by the peak area of 8.654 (Pentadecanoic acid, 14-methyl-, methyl ester with Rt 29.479min and n-Hexadecanoic acid with Rt 30.733min) and the lowest percentage content of peak area of 0.111 (Eicosanoic acid with Rt34.312min). Phytocompounds with their retention time (RT), molecular formula and molecular weight (MW) in the methanolic leaf extract are presented in Table 2.

Table 2: Phyto- chemical components in methanolic extract of *T. subulata* Smith

S.No	Rt	Name of the compound	Molecular formula	Mw	Peak area (%)
1	4.151	Morphinan-4,5-epoxy-3,6-di-ol, 6-[7-nitrobenzofurazan-4-yl] amino-	$C_{26}H_{27}N_5O_6$	505	2.780
2	8.611	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	6.598
3	10.842	Benzene, (ethenyloxy)-	C_8H_8O	120	5. 390
4	12.693	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	1.908
5	14.951	Hydroxylamine, O-decyl-	$C_{10}H_{23}NO$	173	2.062
6	15.198	Caryophyllene	$C_{15}H_{24}$	204	2.062
7	17.998	2,5-Difluorobenzoic acid, 3,5-difluophenyl ester	$C_{13}H_6F_4O_2$	270	1.526
8	21.691	3-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324	3.541
9	22.514	12-Oxa[tetracyclo[5.2.1.1(2,6).1(8,11)] dodecan-10-ol, 3-acetoxy-	$C_{13}H_{18}O_4$	238	2.099
10	27.908	4H-Cyclopropa[5',6']benz[1',2':7,8] azuleno[5,6-b]oxiren-4-one, 8-(acetyloxy)-1	$C_{22}H_{30}O_8$	422	4.541
11	28.176	1b,4a-Epoxy-2H-cyclopenta[3,4] cyclopropa[8,9]cycloundec[1,2-b] oxiren-5(1aH)-one	$C_{28}H_{38}O_{11}$	550	5.400
12	28.456	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	5.400
13	29.479	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	8.654
14	30.733	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.654
15	32.650	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	0.571
16	32.751	Methyl 8,11,14-heptadecatrienoate	$C_{18}H_{30}O_2$	278	23.244
17	33.031	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	4.731
18	33.353	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284	4.731

19	33.871	Oleic Acid	$C_{18}H_{34}O_2$	282	4.731
20	34.312	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.111
21	36.776	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	$C_{19}H_{36}O_3$	312	1.661
22	39.955	BIS(2-Ethylhexyl)phthalate	$C_{24}H_{38}O_4$	390	2.862

DISCUSSION

Traditional medicine also known as indigenous or folk medicine comprises medical knowledge systems that developed over generations with in various societies before the era of modern medicine. Traditional medicines are prepared from a single plant or combination of more than one plant. Indian contribution to herbal market and emphasis on novel research is continuously increasing. Phytochemical constituents are responsible for medicinal activity of plant species [19]. Hence, in the present study phytochemical components in methanolic extract of *T. subulata* leaf extract the highest percentage content of peak area of Methyl 8,11,14-heptadecatrienoate (23.244%), Pentadecanoic acid, 14-methyl-,methyl ester (8.654 %), n-Hexadecanoic acid (8.654 %), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (6.598%), 1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa[8,9]cycloundec[1,2-b]oxiren-5(1aH)-one(5.400%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (5.400%) and the lowest percentage content of peak area of Eicosanoic acid (0.111%).

Ebenezer *et al*., [20] reported that the compound of Methyl 8,11,14 Heptadecatrienoate ($C_{18}H_{30}O_2$) is an Aliphatic Fatty acid compound in methanol extracts of *Glycosmismauritiana* and then identified in petroleum and ethanol extracts of *Ipomoea eriocarpa* leaves [21]. Pentadecanoic acid,14-methyl-,methyl ester ($C_{19}H_{34}O_2$)is a Palmitic acid methyl ester and n-hexanedecanoic acid ($C_6H_{22}O_2$) is a Palmitic acid [22]. These three major compounds are contributing the activities like larvicidal, pupicidal and adulticidal[23], Antifungal, Antimicrobial [24], Antioxidant, Hypochloesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Haemolytic, 5-Alpha reductase inhibitor [22].

CONCLUSION

Methyl 8,11,14 Heptadecatrienoate ($C_{18}H_{30}O_2$) component are already reported with Pharmaceutical and Antibacterial properties. Hence the leaf extracts of *T. subulata* Smith. could positively use in the treatment against various ailments for the Herbal Medicine Industry. Further in future, these components can be isolated and pharmacological activity may be studied to determine the traditional use.

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