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Phytochemical evaluation and GC – MS analysis of *thevetia peruviana* leaves extract

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ABSTRACT

Thevetia peruviana, cultivated as an ornamental plant and planted as large flowering shrub, belongs to the family Apocynacea. The aim of this study is to screen the phytochemicals present in the leaves of Thevetia peruviana and further analysis of the components present in it by GC- MS analysis.The leaves were sequetially extracted based on the polarity viz., hexane , acetone and methanol and subjected to phytochemical screening which revealed the presence of bioactive compounds such as alkaloids, flavanoids, cardiac glycosides, steroids, terpenoids, tannins, phenols, quinones and saponins.The GC- MS analysis of the acetone extract revealed the presence of 33 compounds. This study forms a basis for the biological characterization and importance of the compounds identified.

Introduction

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like leaves, roots, bark, flowers, seeds etc. Plant products provide crucial, unmatched chemical diversity to modern drug discovery programs. The role of traditional medicine in the solution of health problems is invaluable on a global level.The use of herbal medicines have become popular due to the belief that green medicines are safe, easily available with less or no side effects. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today, face either extinction or loss of genetic diversity. Knowledge of the chemical constituents of plant is desirable because such information will be valuable for the synthesis of complex chemical substances and also helps to find the scientific basis of the therapeutic action of these green medicines.

Thevetia peruviana is native to tropical America, especially Mexico, Brazil and West Indies but now frequently grown throughtout the tropical. It belongs to the family Apocynaceae and commonly known as yellow oleander or luckynut. In its native countries, the plant has been known for more than 2000 years. It grows as a shrub or small tree no taller than 8m. The yellow oleander is cultivated in garden and roadside as ornamental plant. Flowers bloom from summer to fall and is orange – yellow in colour. The tender fruits is deep yellow in colour which turns black when is mature. The leaves show a dark green surface. Mostly all parts of plant i.e. leaves, seed, flower, fruits and root are considered as potential source of biologically active compounds.Various bioactivities of Thevetia peruviana such as anti spermatogenic (Gupta et al, 2011), piscicidal (Singh et al, 2010), anti termite (Gachanja et al, 2010), anti fungal (Raul Brunode Sousa et al, 2003; Chewachong et al, 2010), anti inflammatory (venkataraman et al, 2010), anti diarrhoeal, anti microbial and cytotoxic (Ahmed et al, 2011) have been reported.

The aim of the present work was to identify the phytocomponents present in the hexane, acetone and methanol extracts of the leaves of *Thevetia peruviana* by qualitative phytochemical testing and to identify the compounds present in the acetone extract of the leaves by Gas Chromatography – Mass spectrum (GC – MS) analysis.

Materials and Methods

Collection of plant materials

Fresh leaves of *Thevetia peruviana* were collected randomly from Jaipur, Rajasthan and identified and authenticated in the herbarium unit of Department of Botany, Rajasthan University and a voucher specimen (RUBL211530) has been deposited.

Plant extract preparation

The leaves were properly washed with tap water and then rinsed with distilled water. The rinsed leaves were shed dried for 1 week. The crispy leaves were then reduced to powder using a blender and then stored in air tight bottles. The powdered plant material (50gm × 3) was extracted with Hexane, Acetone and Methanol by continuous hot extraction using soxhlet appratus at a temp. not exceeding the boiling point of the solvents. The extacts were then filtered and evaporated on a water bath at low temp. (40˚C - 50˚C) to a syrupy consistency and then to dryness; and were stored at 4˚C until used.

Phytochemical evaluation

The hexane, acetone and methanol crude extracts were subjected to phytochemical tests for the presence of different metabolites using standard protocols viz. Alkaloids (Dragendroff's test, Mayer's test), Flavanoids (Ammonia test), Cardiac glycosides (Killer – Killani's test), Steroids (Salkowski test), Terpenoids (Salkowski test), Tannins (Ferric chloride test), Saponins (Foam test), Phenols (Ferric chloride test) and Quinones (Conc. Sulphuric acid test).

GC – MS analysis

A shimadzu QP – 2010 plus with thermal desorption system TD20 was used to obtain the chromatograms. The name and specification of the column used is Rtx – 5MS (30m×0.25mm internal diameter×0.25µm film thickness).

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Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1.2ml/min., and the sample injected was 2µl in volume. The oven temperature was progammed from 80˚C to 250˚C with constant rise of 5˚C/min. and then held isotermal at 250˚C for 10min.; further the temp. was increased by 30˚C/min. upto 310˚C and again held isothermal at 320˚C for 22min. The injector and ion source temp. were 270˚C and 230˚C respectively. Mass spectra were taken at 70eV; a scan interval of 0.5s and the total GC – MS running time was 45min.

Identification of compounds was based on comparison of their mass spectra with those of NIST (National Institute Standard and Technology) and WILEY libraries mass spectral database.

Results

Phytochemical evaluation

Investigation on the phytochemical screening of hexane, acetone and methanol extracts of leaves of *Thevetia peruviana* revealed the presence of Alkaloids, Flavanoids, Cardiac glycosides, Steroids, Terpenoids, Tannins, Saponins, Phenols and Quinones.The results are presented in Table 1.

GC – MS Analysis

The GC – MS chromatogram (Fig.1) of acetone extract of leaves of *Thevetia peruviana* revealed the presence of thirty three compounds which belongs to different classes of secondary metabolites. The peak report of the chromatogram obtained with details of peak number, retention time, area percentage, name of the identified compound, its moleclar formula and molecular weight are presented in Table 2.

Table 1. Phytochemical Evaluation of leaves extracts of Thevetia peruviana.

NO	Phytochemical Constituents	Name of the test	Hexane	Acetone	Methanol
1	Alkaloids	Dragendroff's Test Mayer's Test	- _	+ _	+++ +++
2	Flavanoids	Ammonia Test	+	+	++
3	Cardiac Glycosides	Kellar Killani's Test	+	+	++
4	Steroids	Salkowski's Test	-	-	-
5	Terpenoids	Salkowski's Test	+	+++	+++
6	Tannins	Ferric chloride Test	-	-	++
7	Saponins	Foam Test	_	+	++
8	Phenols	Ferric chloride Test	+	+	++
9	Quinones	Conc. Sulphuric acid Test	+	+	++

(-) = absent (+) = present (++) = moderately present (++++)=appreciable amount.

Table 2 . Peak report of GC-MS.

Peak	R.time	Area%	Compound Name	Mol. Formula	Mol. Wt.
1	6.894	1.32	Hydroquinone	C6H602	110
2	7.737	1.16	2-Methoxy-4-vinylphenol	C9H1002	150
3	10.006	0.93	4-Benzyloxynitrobenzene	C13H11NO3	229
4	15.345	2.53	2(4H)-Benzofuranone, 5,6,7,7A-Tetrahydro-6-Hydroxy-4,4,7A-trimethyl-,(6S-CIS)-	C11H16O3	196
5	16.168	2.59	2,6,10-trimethyl,14-ethylene-14-pentadecne	C20H38	278
6	16.532	1.51	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296
7	16.797	2.54	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296
8	17.751	1.26	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	C18H28O3	292
9	17.987	1.37	Palmitic acid	C16H3202	256
10	18.090	1.96	Ethyl 9-hexadecanoate	C18H34O2	282
11	18.369	9.12	Ethyl hexadecanoate	C18H36O2	284
12	19.081	3.31	Trimethylsilyl palmitate	C19H40O2Si	328
13	19.750	0.76	Linoleic acid	C18H32O2	280
14	19.841	1.67	Linolenic acid	C19H32O2	292
15	20.006	11.01	Phytol	C20H40O	296
16	20.617	5.70	Ethyl linoleate	C20H36O2	308
17	20.683	14.65	Ethyl oleate	C20H38O2	310
18	20.996	4.16	Ethyl steareate	C20H40O2	312
19	21.303	1.83	Monotrimethylsilyl oleic acid	C21H42O2Si	354
20	23.119	0.46	9-Octadecenamide	C18H35N0	281
21	23.486	1.07	Bis(2-ethylhexyl) adipate	C22H42O4	370
22	25.555	4.71	Bis(2-ethylhexyl) phthalate	C24H3804	390
23	30.495	0.53	Squalene	C30H50	410
24	31.360	2.16	Pentacyclo[19.3.1.1(3,7).1(9,13).1(15,19)]octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaene-25,26,27,28-tetrol, 5,11,17,23-tetral	C44H56O4	648
25	31.573	1.16	Cholesta-3,5-Diene	C27H44	368
26	32.464	1.01	3-Methoxycholest-5-ene	C28H48O	400
27	33.107	3.33	Cholesteryl chloroformate	C28H45ClO2	448
28	33.611	1.73	Stigmasta-5,22-dien-3-yl acetate	C31H50O2	454
29	33.989	1.00	Stigmast-5-en-3-ol, (3.BETA.)-	C29H50O	414
30	34.570	6.86	Stigmast-5-en-3-ol, Oleat	C47H82O2	678
31	35.184	3.13	Vit. E	C29H50O2	430
32	41.809	1.88	Betulin	C30H50O2	442
33	42.032	1.59	4-(1,3,3-Trimethyl-bicyclo[4.1.0]hept-2-yl)-but-3-en-2-one	C14H22O	206

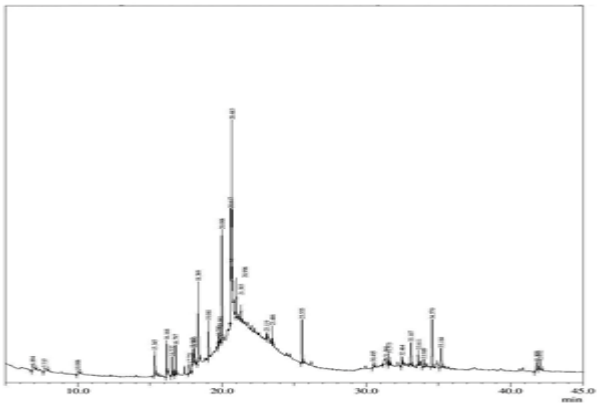

Figure 1. GC- MS Chromatogram.

Discussion

In the present investigation initial screening of the experimental plant leaves using hexane, actone and methanol as organic solvents was done for the presence of different secondary metabolites .Hexane extract showed the presence of flavanoids, cardiac glycosides, terpenoids, phenols and quinones in trace amount while alkaloids and saponins were absent. On the other hand , Acetone extract showed the presence of flavanoids, cardiac glycosides, saponins, phenols and quinones in trace amount while terpenoids in appreciable amount. Methanol extract showed the presence of flavanoids, cardiac glycosides, tannins, saponins, phenols and quinones in moderate amount and alkaloids and terpenoids in appreciable amount. Flavanoids and alkaloids are known to possess general antimicrobial activity. Tannins possess antibacterial activity by damaging bacterial cell membrane. Cardiac glycosides are heart stimulant.

The present study even deals with the GC – MS analysis of acetone extract of leaves of the experimental plant .The chromatogram analysis showed thirty three paeks indicating the presence of thirty three compounds in the extract.On comparison of the mass spectra of the compounds with the NIST and WILEY libraries mass spectral database, the thirty three compounds were identified. The most abundant compounds found in this study were Ethyl oleate (Rt = 20.68), Phytol (Rt = 20.00), Ethyl hexadecanoate (Rt = 18.36), Stigmast-5-en-3-ol,oleat (Rt = 34.57) and Ethyl linoleate (Rt = 20.61) which accounted for approximately 14.65%, 11.01%, 9.12%, 6.86% and 5.70% peak area respectively. Many of the compounds identified in the GC-MS analysis have significant reported bioactivities such as Phytol,a diterpene, has cancer – preventive properties; Betulin, a triterpene, has anti-inflammatory, anti-tumor and prostaglandin synthesis inhibitory properties; Hydroquinone, a phenolic compound, has antibacterial, antioxidant and antimelasmic properties; Vit. E, a fat soluble vitamin has antioxidant and anticancer properties; Squalene, a triterpene, has antibacterial, Immuno stimulant and lipoxygenase inhibitory properties; Linoleic acid, a omega fatty acid, has antiarthritic, antihistamic and antieczemic properties.

Conclusion

From the GC-MS profile of the plant leaves, it is evident that a large number and range of phytochemicals like terpenoids, flavanoids, phenols, quinones, saponins, fatty acids and their esters, hydrocarbons and nitrogen and silicon containing compounds are present each of which have its own importance. Presence of such a wide range of phytochemicals provides a scope for the further investigation of these compounds for various pharmacological properties and their use as a potent drugs in near future.Therfore this study is a step towards exploration of novel compounds in leaves extracts of *Thevetia peruviana.*

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