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GC-MS analysis of various solvent extracts of bark in *Solanum verbascifolium* Linn.

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ABSTRACT

The present investigation was carried out to determine the essential chemical constituents in the bark of *Solanum verbascifolium* Linn. A total of 21 phytocompounds were identified in three different extracts from the bark extract of the plant. Among these 13 constituents in ethyl acetate, 6 in methanolic and 2 in acetone extract were identified during the GC-MS analysis. Phytol and Linolenic acid which were identified in the plant is considered to have anti-cancerous properties.

Keywords: Ethnomedicine, Kurumba, Kundah, Nilgiri.

INTRODUCTION

In the present scenario, the understanding of the chemical constituents of plants with medicinal properties supports not only to pave way for new drug discovery but also play a crucial role in identifying new source of economically viable phytocompounds[1]. The study also helps in validating the actual significance of the traditional medicinal practices. Hence thorough understanding of these chemical constituents became the key focus area in standardizing the natural drug because many of these phytocompounds exhibits a complementary and overlapping mechanism of activity. Mass spectrometry, along with chromatographic separations techniques such as Gas chromatography (GC/MS) are usually used in case of direct analysis of the components existing in traditional medicinal practices and medicinal plants[2]. In recent years GC-MS studies have been increasingly adopted for the analysis of medicinal plants because this technique has proved to be an effective method for the analysis of non polar components and volatile essential oil, fatty acids, lipids and alkaloids[3].

Solanum verbascifolium Linn. is a perennial small tree with dense stellately tomentose hairs growing up 6-7 m in height belongs to family Solanaceae. It is a woody and shrubby plant found throughout the Nilgiris. Leaves subopposite entire, lobed velvety tomentose, tawny beneath, elliptic lanceolate. Flowers with terminal corymbose cymes, corolla white; calyx is greyish short lobed and cup shaped. Fruit berry yellow, globose, with few stellate

hairs, entire. Seeds minutely papillose scaly with fleshy albumen. In Kurumba dialect name of the plant is known as "Pithemaram"

Medicinal importance of the *Solanum verbascifolium*

From the literature review the plant possess an important place in the folklore medicine. According to Chinese folklore medicinal knowledge the decoction of roots taken internally for diarrhoea and dysentery, eczema, edema, gout, antioxidant property and toothache[4]. In Mexico, leaves are heated and applied to forehead for headaches; and as poultice, to boils and ulcers. In Nayarit, decoction of roots used for fever, decoction of root with piece of *Zingiber officinalis* rhizome and *Alium cepa*, used in the treatment of hematuria, Leaves are used for the expelling of impurities through the urine; used in case to treat women with vaginal discharge, Leaves are also used as abortifacient[5]. Studies have also verified that the plant contains highly beneficial contents, like protein, high amounts of carbohydrates, potassium, iron, sodium, calcium and phosphorous, Vit. A and Vit. C which can be exploited by healthcare industries as nutritional supplements[6]. The plant extracts has also proved to be a antibacterial agent[7].

The aim of the present investigation is to identify the biochemical components of *Solanum verbascifolium* by subjecting methanolic, ethyl acetate and acetone extracts of the stem bark to Mass spectrum analysis (GC-MS analysis).

MATERIAL AND METHODS

According to the Kurumba folklore knowledge for curing gastro-intestinal infection, the stem bark infusion of *Solanum verbascifolium* is orally consumed at regular intervals. Therefore the stem bark was collected during the field trip which was carried out in the Kurumba settlement called Belathicombai in Onikandi near Manjoor town in Kundah taluk of Niligiri district during 2009-2010 (Figure 1 and 2). All the collected plant specimens were identified taxonomically with the help of The Flora of Presidency of Madras[8], The Flora of Tamil Nadu Carnatic[9] and The Flora of South Indian Hill Station[10] and the herbarium was prepared by following the procedure described in Methods and Approaches in Ethnobotany[11]. The voucher specimens were deposited at the RIEM herbarium.

Preparation of the stem bark extract

Fresh plant materials (tender bark) of *Solanum verbascifolium* which are free from infections were collected from the study area. The barks were washed thoroughly 2-3 times with running water to remove the soil particles and other dirt. The material was then shade dried on a sterile blotter for 40 days, afterwards in a ventilated oven for 40^0C and subsequently macerated to a powder form by with a mixer grinder and sieved. The powder was stored in air sealed polythene bags at room temperature before extraction. The selection of solvent medium for extraction was done based on the results of the antimicrobial activity. Hence the ethyl acetate, methanol and acetone exhibited remarkable antimicrobial activity and was used as solvents. Then required amount of the sample was weighed and transferred to a Stoppard flask and was treated with the three solvents separately, until the powder was fully immersed and shaken, then incubated overnight. The extracts were then filtered using whatmann filter paper No. 41. Later the extracts were collected and evaporated to dryness with the help of a vaccum distillation unit and the final residue was used for GC-MS analysis.

GC–MS analysis

1µl of the ethyl acetate, methanol and acetone extracts of *Solanum verbascifolium* was used separately for the

carrying out the GC-MS analysis for various phytochemical compounds present in the stem bark of the plant. GC- MS analysis was carried out using a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25mm × ID × 1 µm of capillary column, composed of 100% Dimethyl poly siloxane), operating in electron ionization system with an impact mode of 70 eV was used ; helium (99.998%) was used as carrier gas at a constant flow rate of 1ml/min and an injection volume of 1 µl was employed split less, injector temperature of 250^0C; ion – source temperature 280^0C. The oven temperature was programmed from 40^0C (isothermal for 5 min), with an increase of 10^0C/min, to 300^0C/min isothermal; then hold for 5 mins. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450Da. Total GC run time was 34 mins[12].

Identification of the components

The interpretation of the mass spectrum GC-MS was carried out using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the sample were ascertained using NIST Ver. 2.1 MS data library. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library version[13].

RESULT AND DISCUSSION

GC- MS analysis was carried out on ethyl acetate, methanolic and acetone extracts of *Solanum verbascifolium* stem bark and 21 phytoconstituents were detected. The active principles with their retention time (RT), molecular formula, molecular weight and peak area (%) are presented in Table -1. The ethyl acetate, methanol and acetone extracts chromatograms showed the presence of 13, 6 and 2 major peaks respectively (Figure-3, 4 and 5). The detailed tabulation of the GC-MS analysis of all the 3 extracts revealed that in case of methanol and acetone chromatogram's most of the peaks are of similar nature to that of ethyl acetate extract, except for few phytocompounds which were extracted only in methanol are Formic acid; Trimethylhydroxysilane;1-Naphthoic acid; 1-Piperazineethanamine; Hexadecanoic acid, methyl ester (Figure-4). Similarly in case of acetone only two compounds, 2-Pentanone and gamma-sitosterol were different rest all other peaks were of similar in nature to the peaks of ethyl acetate and methanol extracts (Figure-5).

Among the three extracts the highest peak area (%) of 7.74 was obtained by Trimethylhydroxysilane (Retention time – 5.426) in methanolic extract, then 4.75 was obtained by 2- Pentanone (Retention time-21.259) and the lowest peak area (%) of 0.02 was obtained by 1-Dodecanol (Retention time -20.388) in ethyl acetate extract.

According to the existing literature review eight medicinally important phytoconstituents were identified like gamma-Sitosterol which is used to control hypercholesterolemia[14], Pyridine which is proved effective as CNS depressant[15], and Phytol[16] and Also the plant posses variety of fatty acids like Palmitic and Linolenic acid which are highly medicinal[17]. Detailed medicinal values of various phyto-constituents are tabulated in (Table -2). In the recent years many of the synthetic drugs consumptions resulted in some side effects in the due course of administration. Thus plant based compounds are preferred over the synthetic ones which causes minimal side effects[18]. The presence of these

medicinally important phytocompounds justifies the medicinal value of this plant.

CONCLUSION

The present study of the analysis of three different extracts ethyl acetate, Methanol and acetone has suggested the presence of 21 of phytocompouds. Similarly the methanolic and ethyl acetate extract also showed the presence of 3 and 1 important compound each. Thus the medicinal plant *Solanum verbascifolium* is found to possess significant phytoconstituents. The presence of such variety of phytochemicals may justify the use of the plant in the traditional system of Kurumba medicine for treating gastro intestinal ailments in the form of bark infusion of the plant. Indeed further studies should be carried out to elucidate the main bio active compounds responsible for the activity and thereby it's made available for people at large.

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Table 1. Phyto components identified from the three extracts of *Solanum verbascifolium* linn.

No	Extract	RT	Name of the compound	Molecular formula	MW	Peak area %
1	Ethyl acetate	5.187	Acetamide	C_2H_5NO	59.0672	0.60
2		5.347	Acetohydroxamic acid	$C_2H_5NO_2$	75.0666	0.58
3		5.760	Pyridine	C_5H_5N	79.0999	0.97
4		15.183	5-Tetradecene,(E)-	$C_{14}H_{28}$	196.3721	0.24
5		17.712	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.2372	0.52
6		19.994	1-Nonadecene	$C_{19}H_{38}$	266.5050	0.29
7		20.388	1-Dodecanol	$C_{12}H_{26}O$	186.3342	0.02
8		20.459	Bicyclo3.1.1heptanes, 2, 6, 6-trimethyl-	$C_{10}H_{18}$	138.2499	0.66
9		21.358	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.3975	0.69
10		22.057	Trichloroacetic acid	$C_2HCl_3O_2$	163.387	0.34
11		23.023	9, 12, 15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292.4562	0.66
12		23.178	Phytol	$C_{20}H_{40}O$	296.5310	0.33
13		23.309	Octadecanoic acid, methyl ester	$C_{19}H_{34}O_2$	294.4721	0.22
1	Methanol* Acetone*	5.281	Formic acid, 1 methylethyl ester	$C_4H_8O_2$	88.1051	0.32
2		5.426	Trimethylhydroxysilane	$C_3H_{10}OSi$	90.1964	7.74
3		5.859	1-Naphthoic acid	$C_{11}H_8O_2$	172.1800	0.41
4		11.965	1- Piperazineethanamine	$C_6H_{15}N_3$	129.2034	1.57
5		21.347	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	0.41
1		21.259	2-Pentanone	$C_6H_{12}O_2$	116.1583	4.75
2		33.996	Gamma – Sitosterol	$C_{29}H_{50}O$	414.7067	0.51

* In case of Methanol and Acetone only new compounds were listed remaining compounds are same as Ethyl acetate extract

Table 2. Activity of phytocompounds identified in various extracts of the bark of *Solanum verbascifolium* linn.

S. No.	Name of compound	Compound nature	** Activity
1	Gamma - Sitosterol	Sterols	To treat hypercholesterolemia
2	Pyridine	Heterocyclic compound	CNS –depressant
3	Phytol	Terpenes	Anti cancerous
4	Hexadecanoic acid, methyl ester	Palmitic acid ester	To treat hypercholesterolemia and Antiandrogenic
5	Octadecanoic acid, methyl ester	Palmitic acid ester	Antialopecic, Antioxidant and Antifibrinolytic
6	9,12,15-Octadecatrienoic acid, methyl ester	Linolenic acid	Antiacne, Antialopecic, Antianaphylactic, Antiandrogenic, Antiarteriosclerotic, Antiarthritic, Anticoronary, Antieczemic, Antifibrinolytic, Antigranular, Antihistaminic, Antiinflammatory, Antimenorrhagic, Antiprostatitic, Cancer-Preventive, Hepatoprotective, Hypocholesterolemic, Immunomodulator, Comedolytic
7	1-Naphthoic acid		Cataractagenic
8	Formic acid, 1 methylethyl ester	Ester	Antiseptic, Antisyncopic, Astringent

**Source: Dr. Duke's phytochemical and Ethnobotanical databases online databases

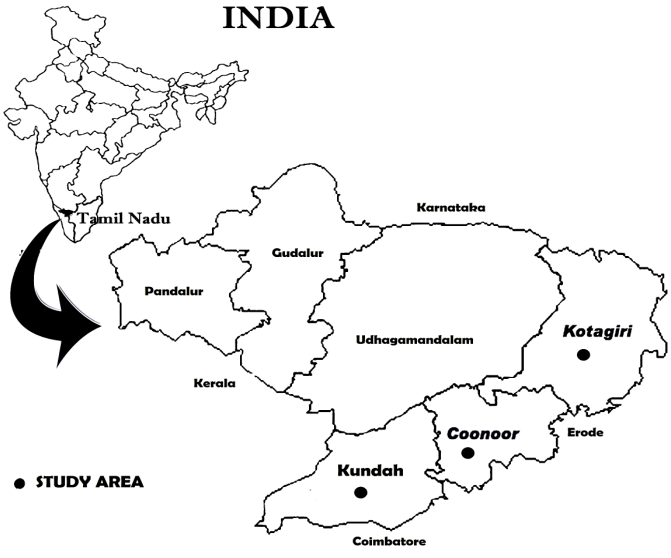

Figure 1. Study area

(A) **(B)** **(C)**

Figure 2. A. Plant in the habitat, B. Collection of the bark, C. Kurumba healer along with the medicinal plant in the habitat

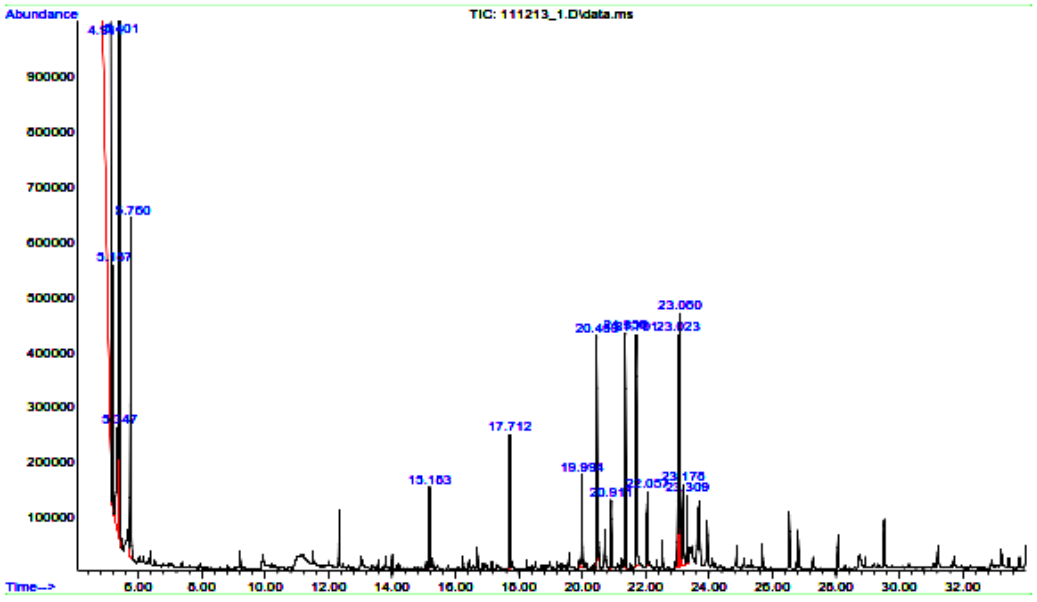

Figure 3. Chromatogram of ethyl acetate bark extract of *Solanum verbascifolium* by GC-MS

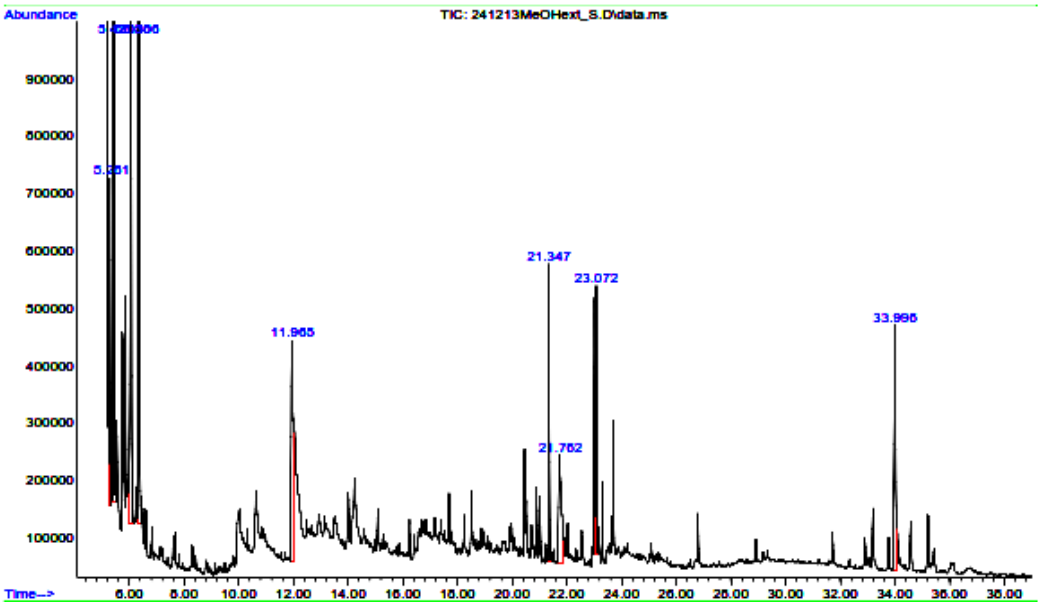

Figure 4. Chromatogram of methanol bark extract of *Solanum verbascifolium* by GC-MS

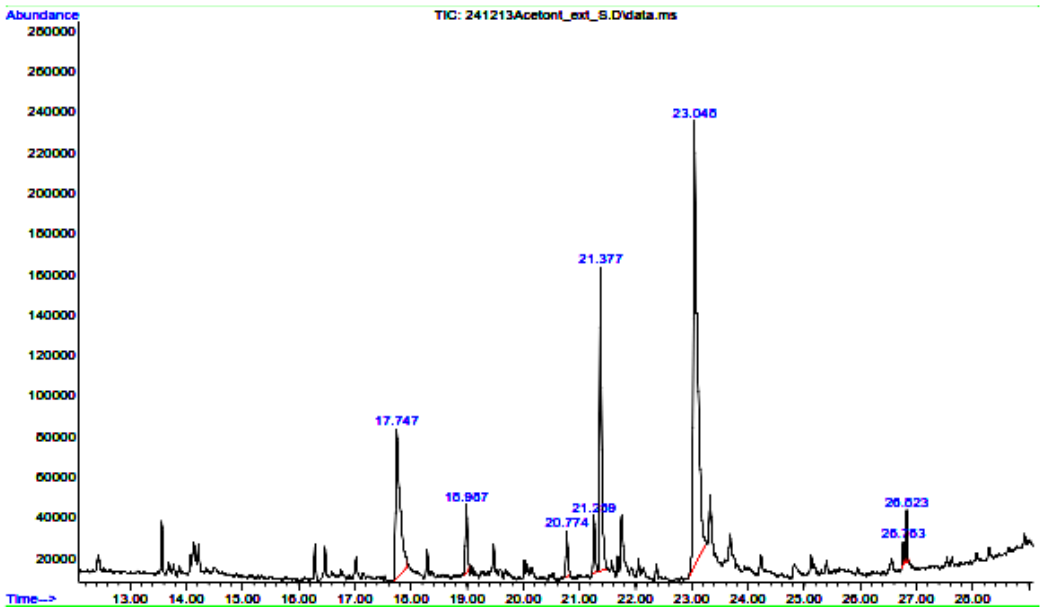

Figure 5. Chromatogram of acetone bark extract of *Solanum verbascifolium* by GC-MS

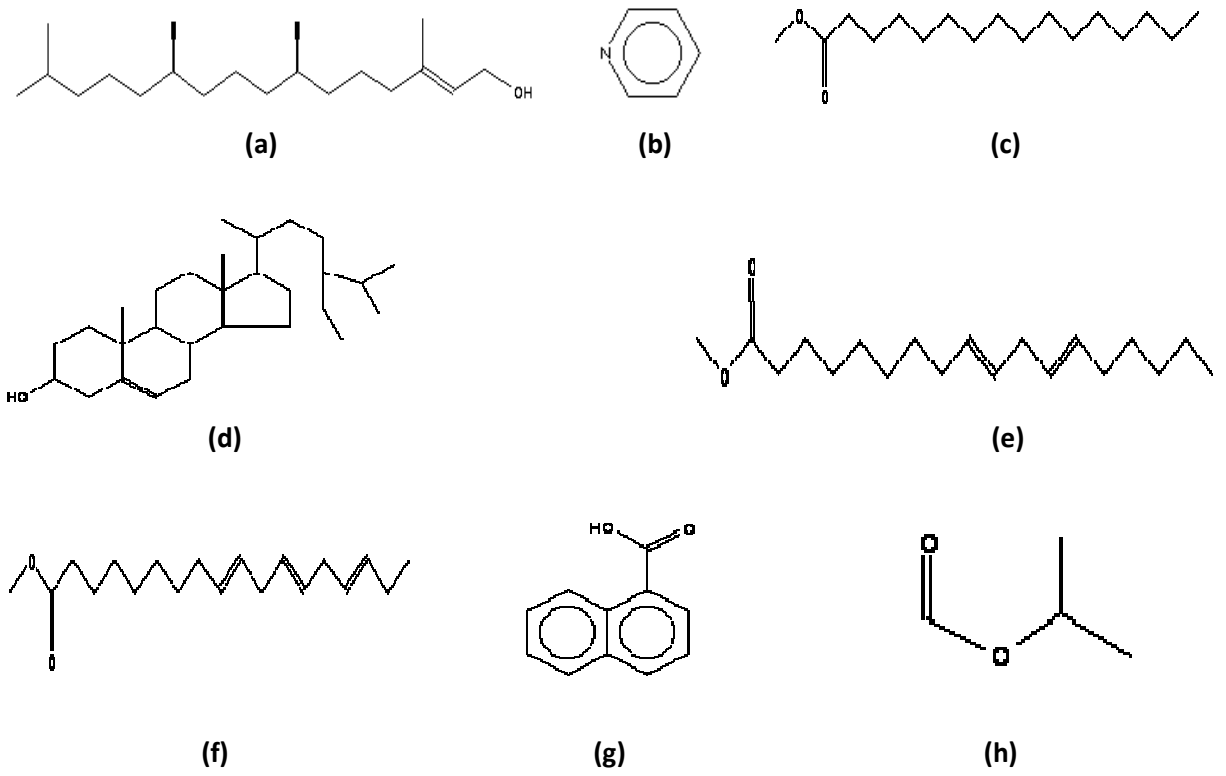

Figure 6. Structural depiction of the important phytocompounds: **a-** Phytol, **b-** Pyridine, **c-** Hexadecanoic acid, methyl ester, **d-** gamma–Sitosterol, **e-** Octadecanoic acid, methyl ester, **f-** 9, 12, 15-Octadecatrienoic acid, methyl ester, **g-** 1-Naphthoic acid, **h-** Formic acid