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Phytochemical profile of *Berberis tinctoria* Lesch. bark using GC-MS analysis

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ABSTRACT

Berberis tinctoria Lesch. is an evergreen species endemic to the high hills of Nilgiris and has high medicinal value. The present study was carried out to understand the phytochemical constituents of the bark part. The results of the GC-MS analysis carried out in three different extracts viz Acetone, Methanol and Ethyl acetate which exhibited the presence of 22 major phytocomponents. The study also highlighted the presence of some known biologically active phytocomponents like berberine, phytol and stigmasterol etc. Hence present study will further validate the medicinal potentiality of this plant.

Keywords: Ethnomedicine, Kurumba, Kundah, Nilgiri.

INTRODUCTION

In the present context, greater focus have been laid on the aboriginal knowledge in the area of bioprospecting of biological resources as a new source of drugs, medicine, food and other industrial uses. At the same time the existing synthetic drugs which exhibit severe undesirable side effects and also less effectiveness of these drugs against the new disease causing bacterial strains have brought in a huge demand for these green medicinal sources. Medicinal plants form a rich source of antibacterial agents. Hence a wide range medicinal plant parts are used for extract as a powerful drug and they possess varied medicinal properties [1].The plants used in ethnomedicinal practises contain a wide range of substance that can be used to cure various chronic as well as infectious diseases. Most of these plants are rich in secondary metabolites and essential oils of therapeutic importance [2, 3].

Berberis tinctoria Lesch. an evergreen erect shrub with yellow wood belonging to Berberidaceae. It is also commonly called as Nilgiri Barberry (Kurumba dialect name: Jakkala) and is found in the inner shola forest of the Nilgiri and Palani hills, at an altitude of 1,800 m [4]. Leaves simple, fascicled in the axils of 3-5 partite with simple spines. Flowers yellow, solitary fascicled racemose corymbose with 2-3 small appressed bracteoles. Red berries of glaucous spindle – shape with short stout styles and few seeded. The plant contains an important alkaloid berberine which is isolated from Berberis species have proven effective for various infectious diseases and also possess antibacterial property [5]. The leaves of *B. tinctoria* have been evaluated for hepatoprotective activity and antioxidant activity [6]. The use of berberine has been described in Indian and Chinese medicine for the treatment of diarrhea and intestinal parasitic infections [7].

Due to the medicinal importance of the berberine, various researchers carried studies on the quantification of berberine from *B. tinctoria* and *B. aristata* using the HPLC method [8, 9, 10]. Henceforth with this background in the present study, an attempt has been carried out to determine the phytochemical constituents from *B. tinctoria* stem bark using GC-MS in three different extracts acetone, methanol and ethyl acetate respectively.

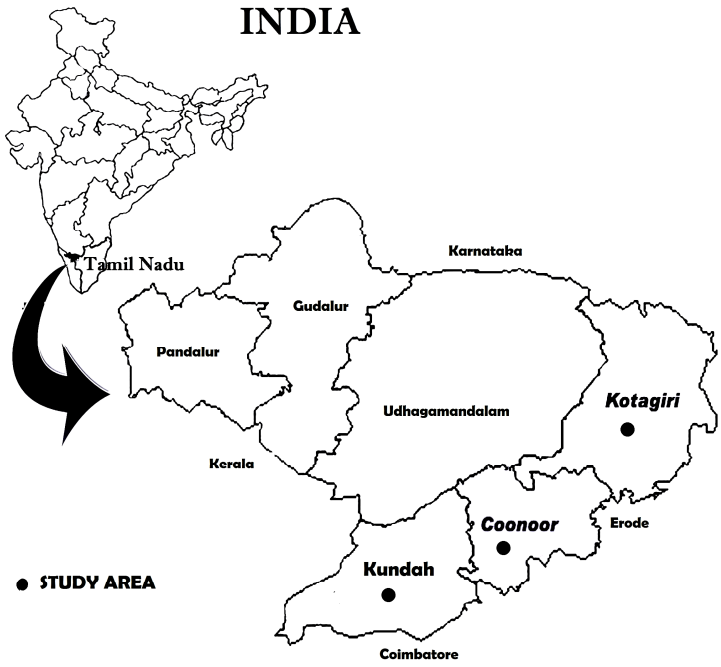


Figure1: Study area

Figure 2

A. Kurumba traditional healer collecting the bark of *B. tinctoria*
B. Cut portion of the bark
C. Bark infusion prepared by dipping the bark in glass of water

MATERIALS AND METHODS

Plant material

Based on the Kurumba folkloric knowledge for curing gastro intestinal infection, the bark infusion of *B. tinctoria* is orally consumed at regular intervals. Therefore the plant stem bark was collected during the ethnobotanical exploration 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 [11], The Flora of Tamil Nadu Carnatic [12] and The Flora of South Indian Hill Station [13] and the herbarium was prepared by following the procedure described in Methods and Approaches in Ethnobotany [14]. The voucher specimens were deposited at the RIEM herbarium.

Preparation of the stem bark extract

Fresh plant materials (tender bark) of *B. tinctoria* which are free from diseases were collected from the study area. The barks were washed thoroughly 2-3 times with running water and one's with sterile distilled water. The material was then shade dried on a sterile blotter for 40 days, afterwards in a ventilated oven for 40^0C and subsequently milled to a fine powder by means of a blender and sieved. The selection of solvent medium for extraction was done based on the results of the antimicrobial activity. Hence the acetone, methanol and ethyl acetate proved remarkable

antimicrobial activity and was used as solvents. Then required quantity of the sample was weighed and transferred to a Stoppard flask and then treated with the three solvents separately, until the powder was fully immersed and shaken and then incubated overnight. The extracts were then filtered using Whatmann filter paper No. 41. Later the extracts collected and evapourated to dryness by using a vaccum distillation unit and the final residue was used for GC-MS analysis.

GC – MS analysis

1µl of the acetone, methanol and ethyl acetate extracts of *B. tinctoria* 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 autosampler 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 2500C; ion – source temperature 2800C. The oven temperature was programmed from 400C (isothermal for 5 min), with an increase of 100C/min, to 3000C/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.

Identification of the phytochemical constituents

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.

RESULTS AND DISCUSSION

The GC- MS analysis of stem bark of *B. tinctoria* revealed the presence of a variety of phytocompounds. The active principles with their retention time (RT), molecular formula, molecular weight and peak area (%) are presented in Table -1. The acetone extract chromatogram showed the presence of 14 major peaks (Figure-3). In case of methanol and ethyl acetate chromatogram's upon analysis revealed that most of the peaks are of similar nature to that of acetone extract except for few phytocompounds which were extracted only in methanol are Berberine, 1-Piperazineethanamine, 4-methyl-, Piperazine, Glycerol, tris (trimethylsilyl) ether, Canadine and Benzene,1,2-dimethoxy-4-(1-propenyl)- (Figure-4). At the same time in ethyl acetate only two phytocompounds, Linoleic acid ethyl ester and n- Hexadecanoic acid were different rest all other peaks were of similar in nature to the peaks of acetone and methanol extracts (Figure-5). The nature of the important phytocompunds is sterols, heterocyclic compounds, isoquinoline alkaloids and terpenes (Table -2). From the existing literature review nine compounds were reported to be medicinally important like β- Sitosterol which is used to control hypercholesterolemia [15], Pyridine which is proved effective as CNS depressant [16], Phytol [17] and Lanosterol has shown anti cancerous properties [18], Stigmasterol, Taraxasterol [19] and Berberine are effective for anti inflammatory activities [20], reports suggest that Piperazine having antihelmintic activity [21] and (-)-Canadine as antimicrobial and antioxidant activity [22]. Majority of the synthetic drugs cause various side effects. Hence, plant based compounds are preferred over the synthetic ones which causes minimal side effects [23]. The presence of these medicinally important phytocompounds can be attributed to the medicinal importance of this plant.

Table – 1 Phyto components identified from the three extracts of *Berberis tinctoria* Lesch.

S No.	Extract	RT	Name of the compound	Molecular formula	MW	Peak Area %
1	Acetone	5.342	Pyridine	C_5H_5N	79.0999	3.70
2		6.040	3-Hexen-2-one	$C_7H_{12}O$	112.1696	0.67
3		6.752	2-Pentanone,4-Hydroxy-4-methyl-	$C_6H_{12}O_2$	116.1583	62.11
4		17.710	Diethyl phthalate	$C_{12}H_{14}O_4$	222.2372	1.54
5		20.459	Bicyclo [3.1.1] heptane,2,6,6- trimethyl-	$C_{10}H_{18}$	138.2499	2.07
6		21.358	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	3.22
7		23.021	9,12- Octadecadienoic acid (Z,Z) - , methyl ester	$C_{19}H_{34}O_2$	294.4721	2.97
8		23.079	9,12,15 – Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	294.4562	3.67
9		23.182	Phytol	$C_{20}H_{40}O$	296.5310	1.90
10		23.305	Octadecanoic acid, methyl ester	$C_{19}H_{34}O_2$	294.4721	1.00
11		33.202	Stigmasterol	$C_{29}H_{48}O$	412.6908	1.72
12		34.004	β - Sitosterol	$C_{29}H_{50}O$	414.7067	8.63
13		35.220	Lanosterol	$C_{30}H_{50}O$	426.7174	2.08
14		35.440	Taraxasterol	$C_{30}H_{50}O$	426.7174	3.10
1	Methanol	32.969	Berberine	$C_{20}H_{18}NO_4$	336.36126	34.53
2		11.972	1-Piperazineethanamine, 4-methyl-	$C_7H_{17}N_3$	143.23	17.27
3		13.977	Piperazine	$C_4H_{10}N_2$	86.1356	5.29
4		18.654	Glycerol, tris (trimethylsilyl) ether	$C_{12}H_{32}Si_3$	308.6372	1.22
5		30.576	Canadine	$C_{20}H_{21}NO_4$	339.38504	2.49
6		29.884	Benzene,1,2-dimethoxy-4-(1-propenyl)-	$C_{11}H_{14}O_2$	178.2277	1.84
1	Ethyl acetate	23.635	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308.4986	0.34
2		21.714	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	3.17

- *In case of Methanol and Ethyl acetate only new compounds were listed rest all the compounds are same as acetone extract*

Table- 2. Activity of phytocompounds identified in various extracts of the bark of *Berberis tinctoria* Lesch.

S. No.	Name of compound	Compound nature	** Activity
1	β - Sitosterol	Sterols	To treat hypercholesterolemia
2	Pyridine	Heterocyclic compound	CNS –depressant
3	Phytol	Terpenes	Anti cancerous
4	Lanosterol	Sterols	Anti cancerous
5	Stigmasterol	Sterols	Anti hepatotoxic, Anti inflammatory, Antioxidant, Anti viral
6	Taraxasterol	Sterols	Anti-inflammatory, Antiedemic
7	Berberine	Isoquinoline alkaloids	Anti-inflammatory, Stomachic, Anticancer, Analgesic, Antibiotic, Anticholera, Antidysentric, Anti bacterial
8	Piperazine	Heterocyclic compound	Anti helminthic
9	(-)-Canadine	Isoquinoline alkaloids	Anti microbial and Anti oxidant

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

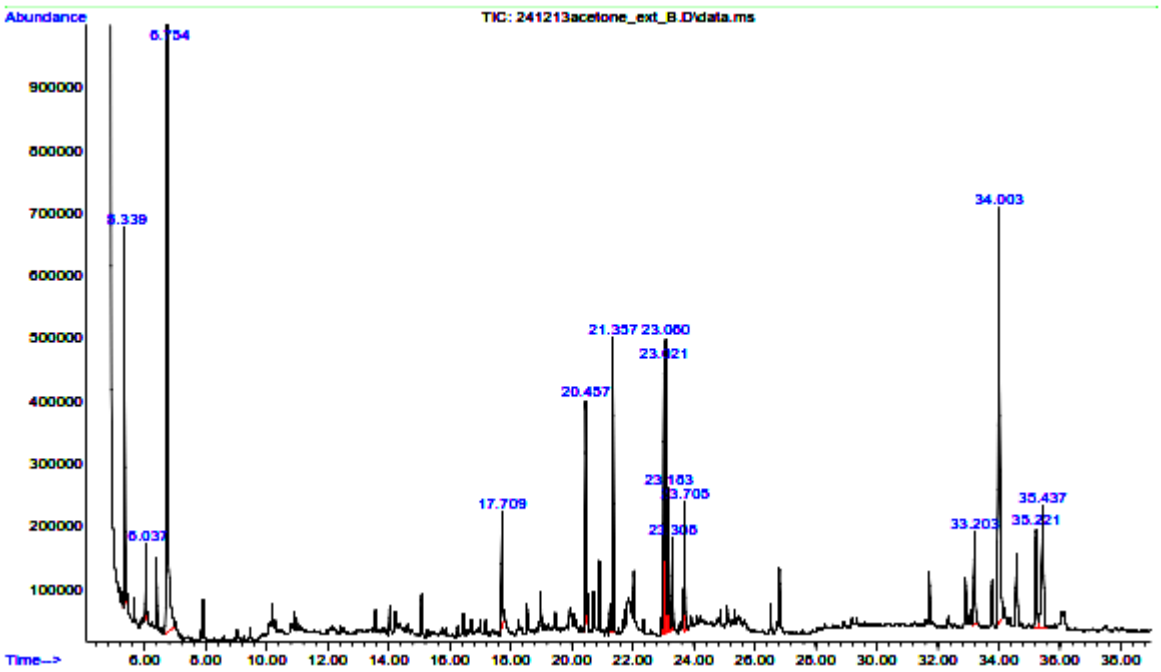


Figure 3: Chromatogram of acetone bark extract of *B. tinctoria* by GC-MS

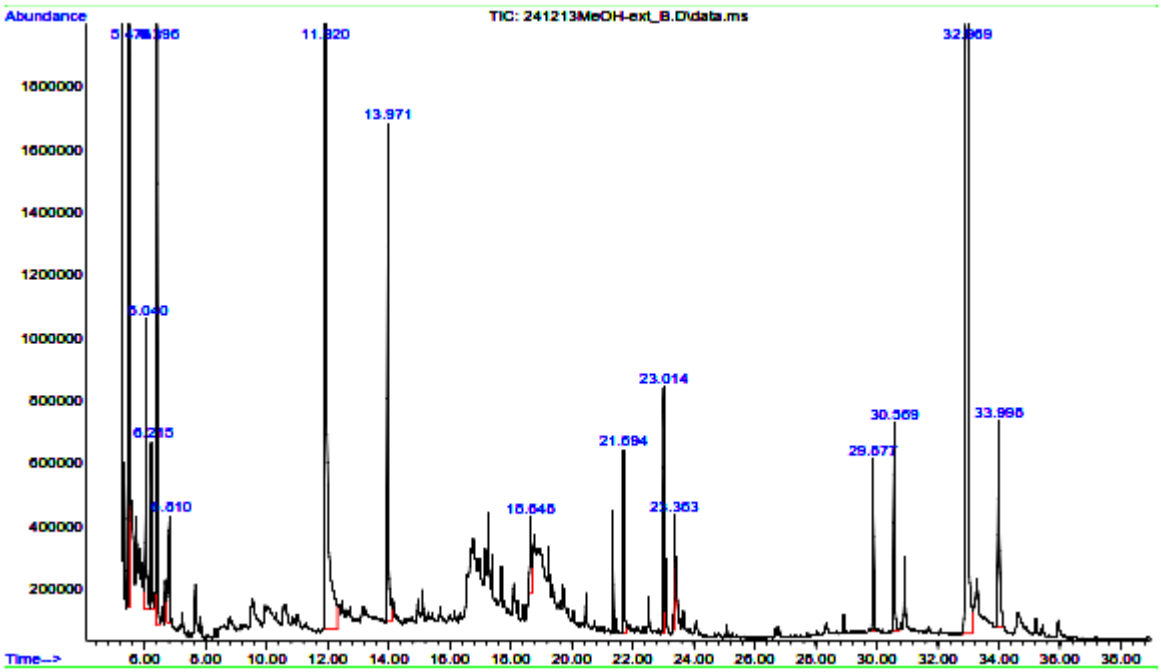


Figure 4: Chromatogram of methanol bark extract of *B. tinctoria* by GC-MS

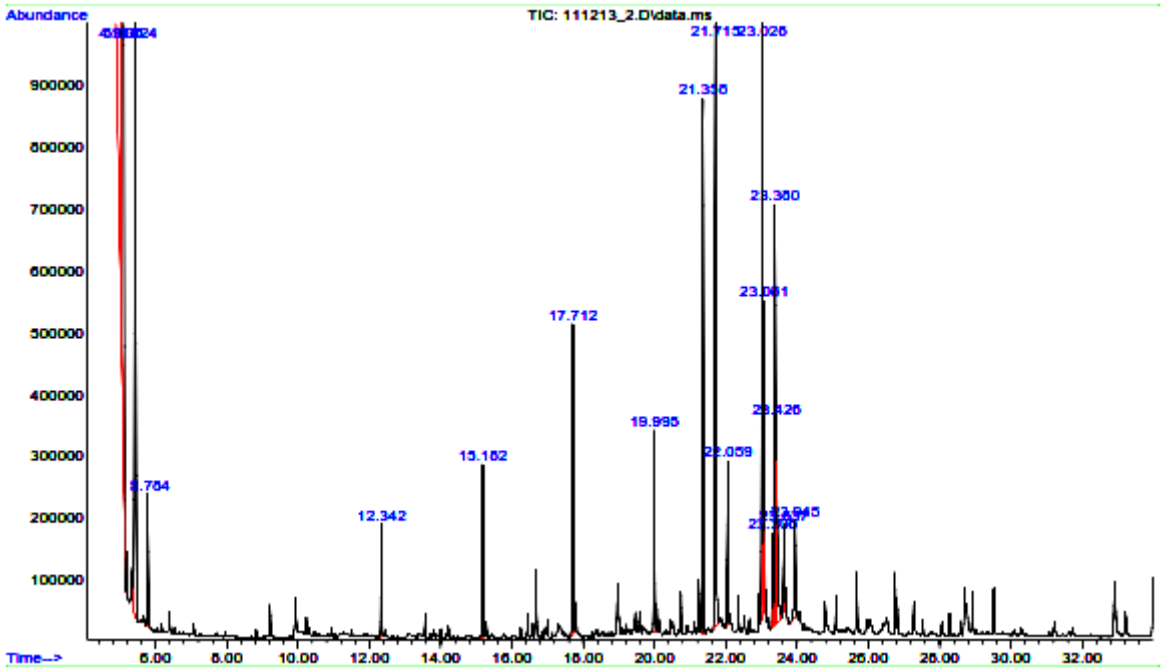
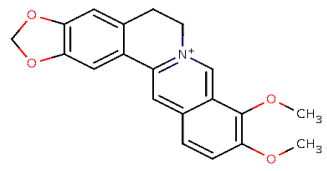
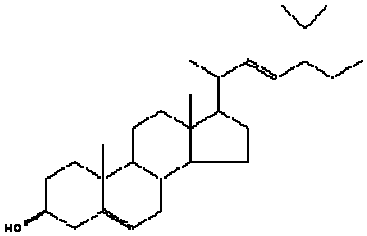


Figure 5: Chromatogram of ethyl acetate bark extract of *B. tinctoria* by GC-MS

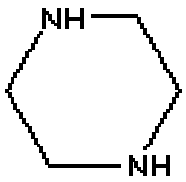
6. a: Berberine



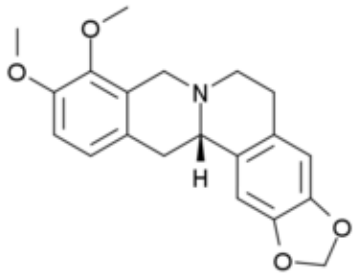
6.b: Stigmasterol



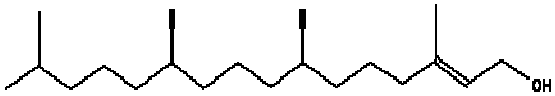
6. c :Piperazine



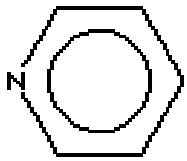
6. d: (-)-Canadine



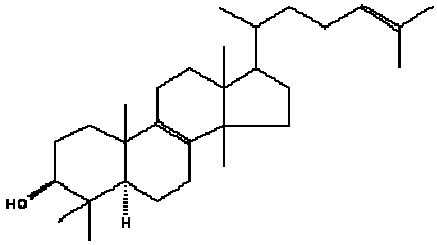
6. e: Phytol



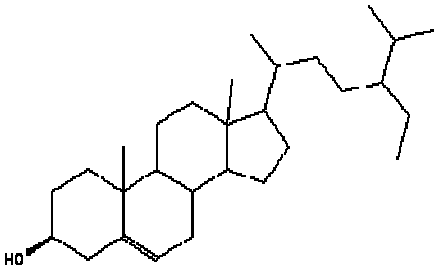
6. f: Pyridine



6. g :Lanosterol



6. h: β – Sitosterol



6. i: Linoleic acid ethyl ester

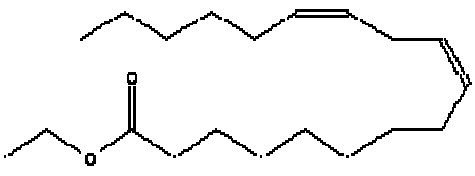


Figure 6: Structural depiction of the major phytocompounds

CONCLUSION

In the current investigation of the three different extracts acetone, Methanol and ethyl acetate have showed the presence of a number of medicinally important compounds. Like in the methanol extracts which exhibited the presence of 6 known therapeutically important compounds. Similarly the methanolic and ethyl acetate extract also showed the presence of 3 and 1 important compound each. Thus the present study justifies the uses of traditional

system of medicines by Kurumbas to treat gastro intestinal disorders using the bark infusion of *B. tinctoria*. However based on these results the plant species can be selected for further investigation for the isolation of this bio active compounds for the development of new potential drugs.

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