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Research Article

Antioxidant Capacity and GC-MS Analysis of Hexane, Ethylacetate and Methanol Extracts of *Ficus bhotanica* – A Potential Folklore Medicinal Plant

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ABSTRACT

Ficus bhotanica is used as one of the important folklore medicine for various ailments by the people of Assam, India and the Ahom community people of this region also used for the preparation of fermentation cake of 'Haanj', the traditional rice based alcoholic beverage. So investigation of this plant was carried out to determine the possible phytochemical components present in the hexane, ethyl acetate and methanol extract of *Ficus bhotanica* and analyse the effective bioactive compounds using GC-MS. Antioxidant capacity of these three extracts of *F. bhotanica* was determined by Deoxyribose assay, Xanthine oxidase inhibitory assay and electrochemical measurements of antioxidant capacity by cyclic voltammetry. Phytochemical screening of hexane, ethyl acetate and methanol extract revealed the presence of alkaloids, saponins, flavonoidsand phenolic compounds. Ethyl acetate extract also shows the presence of tannins. All extracts showed significant antioxidant capacity. The GC-MS analysis revealed the presence of twenty five, eleven and eight major compounds in hexane, ethyl acetate and methanol extract respectively. The presence of the bioactive compounds justifies the use of the plant as medicinal plant.

Keywords: *Ficus bhotanica*, Antioxidant, Radical scavenging, Cyclic voltammetry, GC-MS.

INTRODUCTION

Medicinal plants are the gift from nature to human and have great potential uses, especially as traditional medicine. Traditional medicine based on beliefs and experiences indigenous to different cultures that are used to prevent various diseases, handed down through generations. In both developing and developed countries, because of the scarcity and high costs of orthodox medicine, traditional medicine used as complementary or alternative medicine[1].

Many plants have a variety of phytochemicals and play an important role in the discovery of new drug development for the treatment and prevention of diseases[2]. The most important bioactive constituents of the plants are alkaloids, tannins, flavonoids and phenolic compounds[3,4]. In the recent past, research interest on exploiting biological activities of different medicinal plant has been increasing due to fewer side effects compared to synthetic medicines and it may be due to targeted effect of the phytochemicals on the biochemical pathway[5].

Comprehensive knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in discovering new sources of economic phytocompounds for the synthesis of complex chemical structures and for discovering the actual significance of folkloric remedies[6]. In recent years, GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the identification and quantification purpose by matching the mass spectra of the unknown compound with reference spectra[7].

Jirovetz L. et al. used GC-MS for aroma compound analysis of *Erucasativa* (Brassicaceae) leaf samples and identified more than 50 constituents[8]. Zhao X. et al. analyzed the fatty acids and phytosterols in ethanol extracts of *Nelumbonucifera* seeds and rhizomes by GC-MS[9]. Mayr C. M. et al. used GC-MS for quantitative analysis of 18 aroma compounds related to oxidative off-flavor in wines[10].

Antioxidant is one of the most important topics in recent research and discovering natural antioxidants especially from plant origin due to their beneficial health effects is getting more attention. Antioxidants have the capability to scavenge or deactivate free radicals produced in metabolic process in human body.

F. bhotanica has been extensively used as a traditional medicine by the people of Assam, India especially in diarrhoea, dysentery, cholera, vomiting, joint pain and indigestion. The Ahom community people of Assam also used the leaves of this plant for the preparation of fermentation cakes of "Haanj", the rice based alcoholic beverage.

Taking into consideration of the medicinal importance of this plant, the hexane, ethyl acetate and methanol extract of the leaves of *F. bhotanica*, were analyzed for the first time using GC-MS and antioxidant capacity of this plant was also determined by using Deoxyribose assay,

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Table 1: Classes of phytochemical present in hexane, ethyl acetate and methanol extract of *F. bhotanica*.

S.no	Phytochemical constituents	Hexane extract	Ethylacetate extract	Methanol extract
1	Alkaloids	+	+	+
2	Tannins	-	-	+
3	Phenolic Compounds	+	+	+
4	Flavonoids	+	+	+
5	Saponins	+	+	+
6	Glycosides	-	-	-
7	Quinines	-	-	-

'+'sign indicates present and '-'sign indicates absent

Xanthine oxidase inhibitory assay and electrochemical measurements of antioxidant capacity by cyclic voltammetry.

MATERIALS AND METHODS

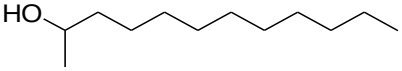
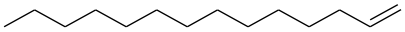
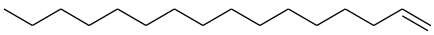
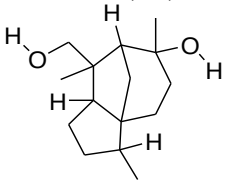
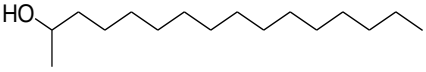
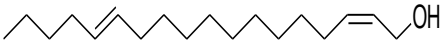
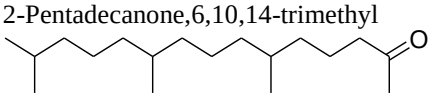
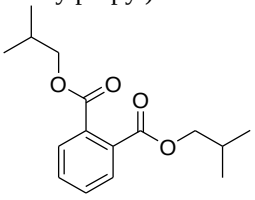
Collection of Plant Material

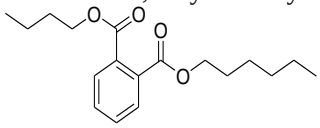
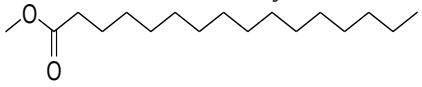
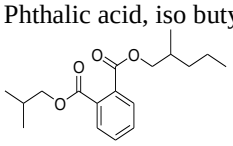
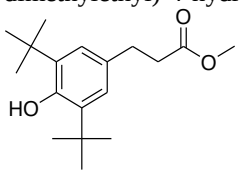
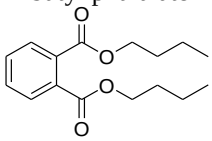
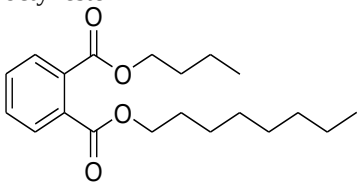
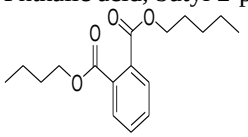
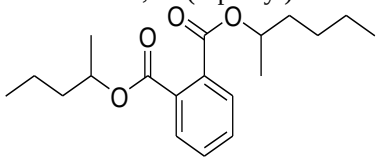
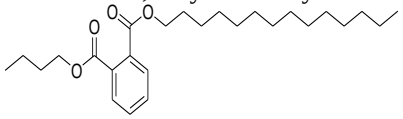
F. bhotanica was collected from Dibrugarh district of Assam, India. The Herbarium of the plant *F. bhotanica* was prepared and the voucher specimen (*F. bhotanica* DCH-32) was preserved in the Department of Chemistry, Dibrugarh University.

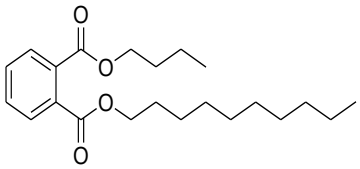
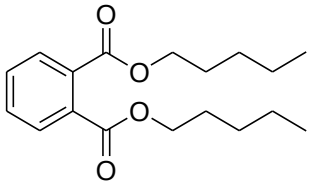
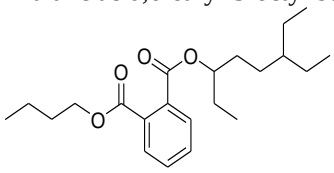
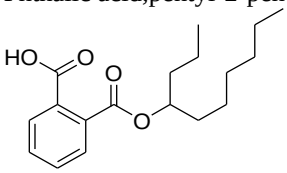
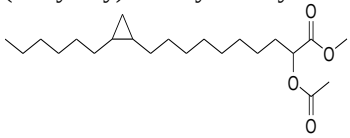
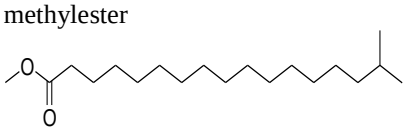
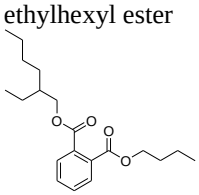
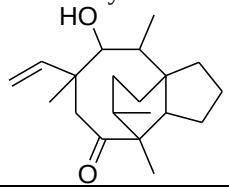
Reagents and Chemicals

The chemicals mercuric chloride, sodium hydroxide, aluminium chloride, sodium potassium tartrate, sodium nitrate, acetic acid, hydrochloric acid, sulphuric acid, nitric acid, potassium hydroxide, hydrogen peroxide, potassium dihydrogen phosphate were purchased from Merck. Sodium carbonate, potassium iodide, iodine, ferric chloride, aluminium chloride, potassium persulphate,

Table 2: Phytochemicals identified in the hexane extract of *F. bhotanica* by GC-MS analysis.

Sl. No	R_t (min)	Name and structure of the compound	Molecular Formula	Molecular weight	Peak area %	Biological activity	Ref.
1	7.746	2-Dodecanol 	$C_{12}H_{26}O$	168	0.76	----	
2	10.487	1-Tetradecene 	$C_{14}H_{28}$	196	3.72	surface active agents, lubricants and lubricant additives	14
3	13.454	1-Hexadecene 	$C_{16}H_{32}$	224	2.88	antibacterial activity	14
4	15.179	Cedran-diol, 8s,13- 	$C_{15}H_{26}O_2$	238	0.23	antimicrobial and anti-inflammatory property	16
5	15.952	2-Hexadecanol 	$C_{16}H_{34}O$	242	1.50	----	
6	16.485	Z,E-2,13-Octadecadien-1-ol 	$C_{18}H_{34}O$	266	0.34	----	
7	16.572	2-Pentadecanone,6,10,14-trimethyl 	$C_{18}H_{36}O$	268	0.81	allelopathic and antimicrobial activity	17
8	16.888	1,2-Benzenedicarboxylicacid,bis(2-methylpropyl)ester 	$C_{16}H_{22}O_4$	278	1.76	----	

9	17.383	Phthalic acid, butyl isohexyl ester 	$C_{18}H_{26}O_4$	306	4.56	----	
10	17.424	Hexadecanoic acid, methyl ester 	$C_{17}H_{34}O_2$	270	0.13	antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant and antiandrogenic	18,19
11	17.596	Phthalic acid, iso butyl-2-pentyl ester 	$C_{17}H_{24}O_4$	292	1.49	----	
12	17.669	Benzenepropanoicacid,3,5-bis(1,1-dimethylethyl)-4-hydroxy,methyl ester 	$C_{18}H_{28}O_3$	292	1.91	antifungal and antioxidant property	18, 20
13	17.877	Dibutyl phthalate 	$C_{16}H_{22}O_4$	278	23.76	antifungal, antimicrobial and antimalarial agent	15, 18
14	17.953	1,2-Benzenedicarboxylicacid butyl octyl ester 	$C_{20}H_{30}O_4$	334	1.35	antimicrobial and antifouling property	21
15	18.086	Phthalic acid, butyl 2-pentyl ester 	$C_{17}H_{24}O_4$	292	15.15	antioxidant and antimicrobial property	22
16	18.302	Phthalic acid, bis(2-pentyl)ester 	$C_{18}H_{26}O_4$	306	1.67	plasticizer	22
17	18.354	Phthalic acid, butyl tetradecyl ester 	$C_{26}H_{42}O_4$	418	0.53	antibacterial activity	15
18	18.415	1,2-Benzenedicarboxylicacid,butyldecyl ester	$C_{22}H_{34}O_4$	362	2.13	----	

							
19	18.611	1,2-Benzenedicarboxylicacid,dipentyl ester	$C_{18}H_{26}O_4$	306	0.59	----	
							
20	18.816	Phthalic acid,6-ethyl-3-octyl butylester	$C_{22}H_{34}O_4$	362	7.12	plasticizer	22
							
21	19.008	Phthalic acid,pentyl-2-pentyl ester	$C_{18}H_{26}O_4$	306	1.67	----	
							
22	19.169	Cyclopropanedecanoicacid,α-(acetyloxy)-2-hexyl-methyl ester	$C_{22}H_{40}O_4$	368	1.32	----	
							
23	19.396	Heptadecanoicacid,1,6-methyl-methylester	$C_{19}H_{38}O_2$	298	0.43	----	
							
24	19.442	1,2-Benzenedicarboxylicacid,butyl-2-ethylhexyl ester	$C_{20}H_{30}O_4$	334	1.16	---	
							
25	19.645	Tricyclo[5.4.3.0(1.8)]tetradecan-6-one,4-ethenyl-3-hydroxy-2,4,7,14-tetramethyl	$C_{20}H_{32}O_2$	304	0.29	----	
							

perchloric acid, disodium EDTA and copper sulphate were purchased from Rankem. Thiobarbituric acid (TBA), trichloroacetic acid (TCA), deoxyribose and ascorbic acid were obtained from Sigma Aldrich. Xanthine, Nitro blue tetrazolium chloride (NBT) and Xanthine oxidase were obtained from Sigma chemical. Hexane, ethyl acetate, methanol, cyclohexane and N,N-dimethyl formamide (DMF) were of AR grade of Rankem.

Preparation of Plant Extract

Plant materials were cleaned and dried in shade. The dried materials was coarsely ground in a mixer and then the plant materials were extracted by soaking in the solvent for three

Table 3: Phytochemicals identified in the ethyl acetate extract of *F. bhotanica* by GC-MS analysis.

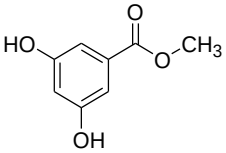
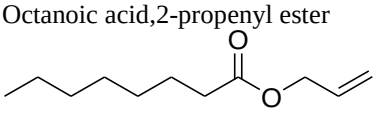
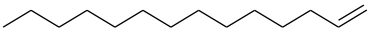
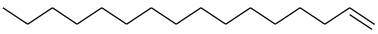
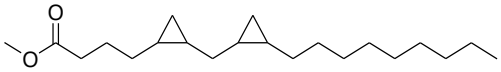
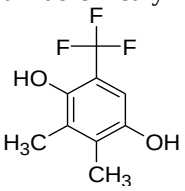
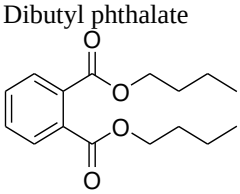
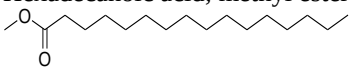
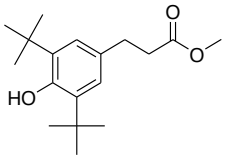
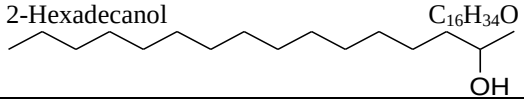
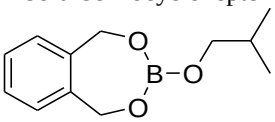
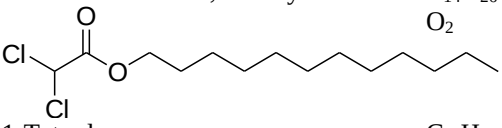
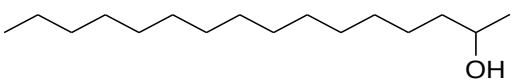
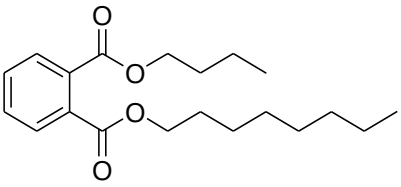
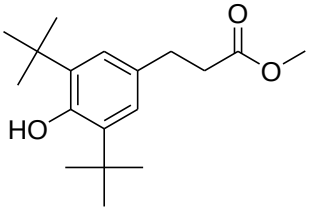
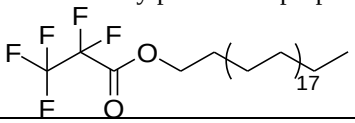
Sl. No	Ret.Time (min)	Name and structure of the compound	Molecular Formula	Molecular weight	Peak area %	Biological activity	Ref.
1	9.401	Benzoic acid, 3,5-dihydroxy, methyl ester 	$C_8H_8O_4$	168	12.05	----	
2	10.309	Octanoic acid,2-propenyl ester 	$C_{11}H_{20}O_2$	184	1.54	----	
3	10.471	1-tetradecene 	$C_{14}H_{28}$	224	12.12	surface active agents, lubricants and lubricant additives	14
4	13.438	1-Hexadecene 	$C_{16}H_{32}$	238	13.59	antibacterial activity.	14
5	15.933	1-Eicosene 	$C_{20}H_{40}$	280	6.26	----	
6	16.467	Cyclopropanebutyricacid,2-[(2-nonylcyclopropyl)methyl]-methyl ester 	$C_{21}H_{38}O_2$	322	1.45	----	
7	16.549	Phen-1,4-diol,2,3-dimethyl-5-trifluoro methyl 	$C_9H_9F_3O_2$	206	1.76	----	
8	16.861	Dibutyl phthalate 	$C_{16}H_{22}O_4$	278	39.97	antifungal, antimicrobial agent and antimalarial.	18
9	17.404	Hexadecanoic acid, methyl ester 	$C_{17}H_{34}O_2$	270	4.15	Antioxidant, hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic.	18,19
10	17.643	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy,methyl ester 	$C_{18}H_{28}O_3$	292	7.57	antifungal and antioxidant property	18, 20
11	18.072	2-Hexadecanol 	$C_{16}H_{34}O$	242	2.43	----	

Table 4: Phytochemicals identified in the methanol extract of *F. bhotanica* by GC-MS analysis.

Sl .No	Ret.Time (min)	Name and structure of the compound	Molecular Formula	Molecular weight	Peak area %		
1	5.627	7-Isobutoxy-5,9-dihydro-6,8-dioxa-7-bora-benzocycloheptene 	$C_{12}H_{17}BO_3$	220	33.81	----	
2	7.740	Dichloroacetic acid, dodecyl ester 	$C_{14}H_{26}Cl_2O_2$	297	3.32	----	
3	10.478	1-Tetradecene 	$C_{14}H_{28}$	224	24.21	surface active agents, lubricants and lubricant additives	14
4	13.444	1-Hexadecene 	$C_{16}H_{32}$	238	17.44	antibacterial activity.	
5	15.942	2-Hexadecanol 	$C_{16}H_{34}O$	242	6.34	----	
6	16.872	1,2-Benzenedicarboxylicacid,butyloctyl ester 	$C_{20}H_{30}O_4$	334	3.22	antimicrobial and antifouling property.	21
7	17.655	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy, methyl ester 	$C_{18}H_{28}O_3$	292	10.43	----	
8	18.080	Octatriacontylpentafluoropropionate 	$C_{41}H_{77}F_5O_2$	696	1.96	----	

days, by using hexane, ethyl acetate and methanol respectively. Plant extracts were concentrated under reduced pressure by using rotary evaporator (Ikon model). Concentrated extracts were stored in desiccators for further studies.

Phytochemical screening[11-12]

Test for Alkaloids

Mayer's test

A fraction of the extract was treated with Mayer's reagent (potassium mercuric iodide solution) and observed for the formation of a yellow coloured precipitate.

Wagner's test

A small amount of the extract was treated with Wagner's reagent (1.27 gm of iodine and 2 gm of potassium iodide in 100ml water) and observed for the formation of reddish brown colour precipitate.

Test for Tannins

Acetic acid test

The extract was treated with acetic acid and observed for the formation of red colour solution.

Braymer's test

Table 5: % inhibition and IC_{50} values of hexane, ethylacetate and methanol extracts of *F. bhotanica* by Deoxyribose assay.

S.no.	Solvent extract	Concentration (µg/mL)	% inhibition of Hydroxyl radical	IC_{50} value (µg/mL)
1	Hexane extract	100	34.745 ± 0.359	190.223 ± 0.547
		200	52.784 ± 0.489	
		300	69.725 ± 0.135	
		400	78.823 ± 0.235	
2	Ethylacetate extract	100	45.176 ± 0.470	133.600 ± 2.734
		200	57.647 ± 0.235	
		300	74.588 ± 0.235	
		400	81.568 ± 0.359	
3	Methanol extract	100	56.235 ± 0.235	37.856 ± 1.071
		200	69.490 ± 0.271	
		300	79.607 ± 0.136	
		400	90.431 ± 0.135	

Table 6: % inhibition and IC_{50} values of hexane, ethylacetate and methanol extracts of *F. bhotanica* by Xanthine Oxidase assay.

S.no.	Solvent extract	Concentration (µg/mL)	% inhibition of Superoxide radical	IC_{50} value (µg/mL)
1	Hexane extract	100	2.820 ± 0.080	398.618 ± 1.726
		200	18.724 ± 0.139	
		300	36.708 ± 0.288	
		400	49.005 ± 0.211	
2	Ethylacetate extract	100	7.775 ± 0.216	357.040 ± 1.509
		200	20.518 ± 0.216	
		300	39.453 ± 0.124	
		400	58.891 ± 0.329	
3	Methanol extract	100	27.670 ± 0.248	217.266 ±1.385
		200	46.308 ± 0.328	
		300	69.964 ± 0.328	
		400	80.143 ± 0.124	

Table 7: E_p values of 1, 4-diaminobenzene alone and in presence of hexane, ethylacetate and methanol extracts of*F.bhotanica*.

Entry	Value of anodic potential of 1,4-diaminobenzene	1^{st} peakE_p(mV)	2^{nd} peakE_p(mV)
1	1,4-diaminobenzene alone	264	910
In presence of plant extracts			
2	Hexane extract of *Ficus bhotanica*	338	-
	Ethyl acetate extract of *Ficus bhotanica*	360	-
	Methanol extract of *Ficus bhotanica*	386	-

2ml of the extract was treated with 10% alcoholic ferric chloride solution and observed for formation of a blue or greenish coloured solution.

Test for Phenolic compounds

Folin-Ciocalteu test

The extract was treated with 10% dilute F-C reagent and 7.5 % aqueous Na_2CO_3 solution and observed for the formation blue colour.

Ferric chloride test

The extract was treated with dilute ferric chloride and observed for the formation of deep blue or violet colour.

Test for Flavonoids

NaOH test

A small amount of extract was treated with aqueous NaOH and HCl and observed the formation of yellow orange colour.

The extract was treated with 5% $NaNO_2$ solutionand then 10% $AlCl_3$ solution. After few minutes 1M NaOH was added to the mixture followed by adding distilled H_2O and observed the formation of pink colour.

Test for Saponins

The extract was vigorously shaken with water and observed for the formation of persistent foam.

Test for glycosides

The extract was treated with Fehling's solution and heated and observed the formation of orange precipitate.

Test for Quinines

The extract was treated with concentrated H_2SO_4 and observed the formation of red colour.

GC-MS Analysis

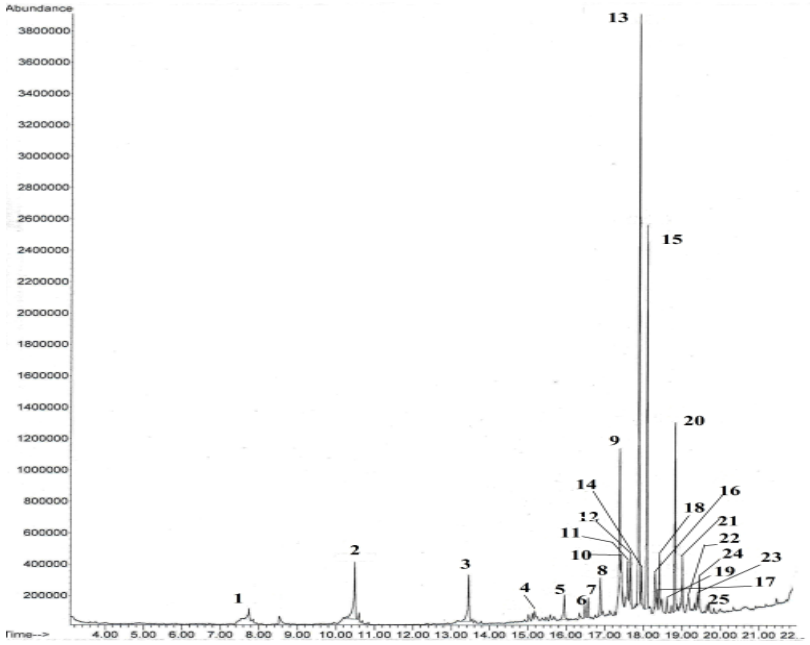


Figure 1: GC-MS Chromatogram of hexane extract of *F. bhotanica*

1. 2-Dodecanol; 2.1-Tetradecene; 3.1-Hexadecene; 4.Cedran-diol, 8s, 13-; 5. 2-Hexadecanol; 6.Z,E-2,13-Octadecadien-1-ol; 7.2-Pentadecanone,6,10,14-trimethyl; 8.1,2-Benzenedicarboxylicacid,bis(2-methylpropyl)ester; 9.Phthalic acid, butyl isohexyl ester; 10.Hexadecanoic acid, methyl ester; 11.Phthalic acid, iso butyl-2-pentyl ester;12.Benzenepropanoicacid,3,5-bis(1,1-dimethylethyl)-4-hydroxy,methyl ester; 13.Dibutyl phthalate; 14.1,2-Benzenedicarboxylicacid butyl octyl ester; 15.Phthalic acid, butyl 2-pentyl ester; 16.Phthalic acid, bis(2-pentyl)ester; 17. Phthalic acid, butyl tetradecyl ester; 18.1,2-Benzene dicarboxylicacid,butyl decylester; 19.1,2-Benzenedicarboxylicacid,dipentyl ester; 20.Phthalic acid,6-ethyl-3-octyl butylester; 21.Phthalic acid, pentyl-2-pentyl ester; 22.Cyclopropanedecanoic acid,α-(acetyloxy)-2-hexyl-methyl ester; 23. Heptadecanoicacid,1,6-methyl-methylester; 24.1,2-Benzenedicarboxylicacid,butyl-2-ethylhexyl ester; 25.Tricyclo[5.4.3.0(1.8)]tetradecan-6-one,4-ethenyl-3-hydroxy-2,4,7,14-tetramethyl

GC-MS analysis on the hexane, ethyl acetate and methanol extract of *F. bhotanica* was carried out in Agilent Technologies GC-MS instrument 7820 A GC system with electron impact ionization (70eV). The specification of the column used was Agilent 19091 S-433: 325°C: 30m × 250 µm × 0.25µm and the column was packed with (5% phenyl)-methylpolysiloxane. Helium was used as carrier gas at constant flow of 1ml/minute. The oven temperature was maintained at 50-280°C at a rate of 10°C/minute. The split ratio was 1:5 and the injector volume was 1µl.

Identification of individual components of the sample was performed by computerized matching of the acquired mass spectra with those stored in Wiley/NIST mass spectral library of the GC-MS data system. The percentage composition of the different components of the sample was calculated from the peak area integrated by the analysis program.

Determination of antioxidant activity

Deoxyribose assay

Hydroxyl radical scavenging activity was measured by the deoxyribose method[13]. Hydroxyl radicals generated by ferric- ascorbate- EDTA-H_2O_2, which attacks on deoxyribose to form products called thiobarbituric acid reactive substances (TBARS), which upon heating with Thiobarbituric acid (TBA) at low pH yield pink chromogen. The hydroxyl radical scavenger, when added, competes with deoxyribose for hydroxyl radical and decreases the TBARS formation and pink chromogen. To the reaction mixture containing 0.36mL 10mM deoxyribose, 0.01mL 10mM ferric chloride, 0.1mL 1mM EDTA, 1mL 1mM ascorbic acid and 0.1mL 10mM H_2O_2 in 0.33mL 50mM Phosphate buffer (pH 7.4) was added 0.1mL (different concentrations 50, 100, 200, 300 µg/mL) of plant extracts. After incubating for 30 min at 37°C, 1mL of this reaction mixture, 1mL of 10% TCA and 1mL of 1% TBA was mixed to yield a final volume 3mL. The reaction mixture was kept in a boiling water bath for 30 min, cooled and the absorbance was measured at 532 nm. Scavenging activities of the plant extracts were expressed as percentage of inhibition of hydroxyl radical.

% inhibition = $[(A_{controle} - A_{sample}) / A_{controle} \times 100]$

Xanthine oxidase inhibitory assay:

According to the method used by Kirby and Schmidt[14] all solutions were prepared in 0.1M Phosphate buffer (pH 7.6). For the control, 1mL of 3mM Xanthine, 1mL of 0.6mM NBT, 0.02mL of 15mM EDTA, 0.5mL of 10mM Xanthine Oxidase and 0.48 mL of 0.1M buffer were mixed. The reaction was incubated at 37°C for 30 min and the absorbance at 560nm was measured using UV-Vis spectrophotometer.

Then scavenging of superoxide anion radical by the plant extracts were monitored by adding 0.1mL (of different

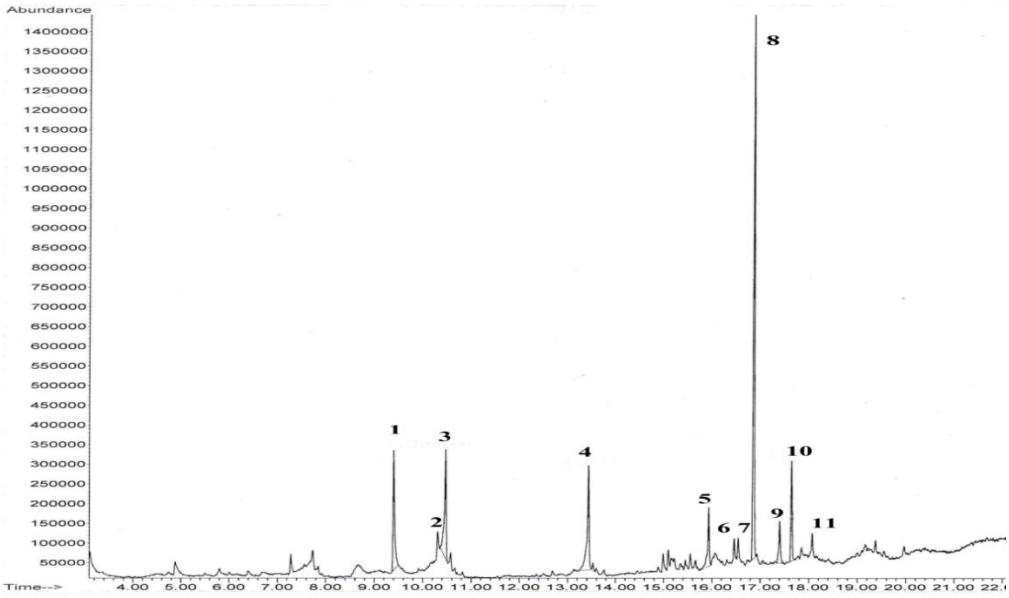


Figure 2: GC-MS Chromatogram of ethyl acetate extract of *F. bhotanica.*
1.Benzoic acid, 3,5-dihydroxy, methyl ester; 2.Octanoic acid,2-propenyl ester; 3.1-tetradecene; 4.1-Hexadecene; 5. 1-Eicosene; 6.Cyclopropanebutyricacid,2-[(2-nonylcyclopropyl)methyl]-methyl ester; 7.Phen-1,4-diol,2,3-dimethyl-5-trifluoromethyl; 8.Dibutyl phthalate; 9.Hexadecanoic acid, methyl ester; 10.Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy, methyl ester; 11. 2-Hexadecanol.

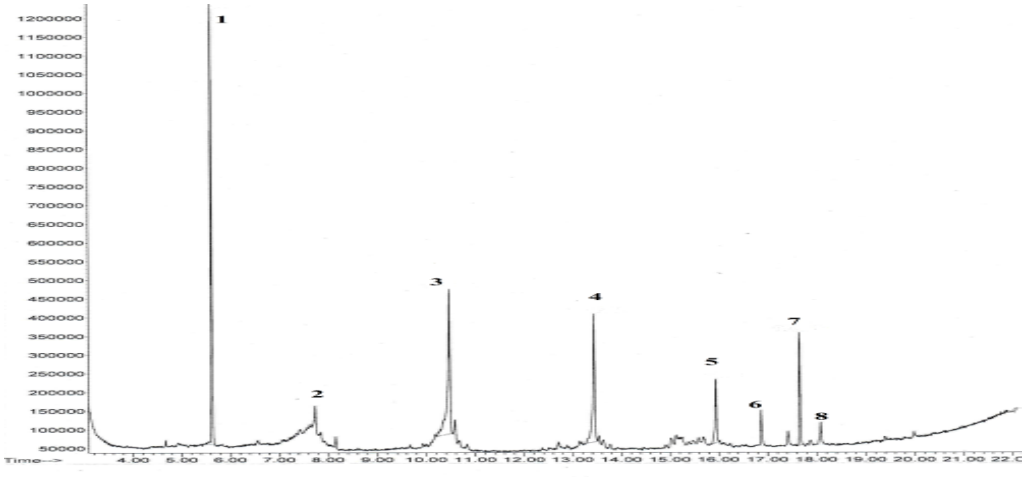
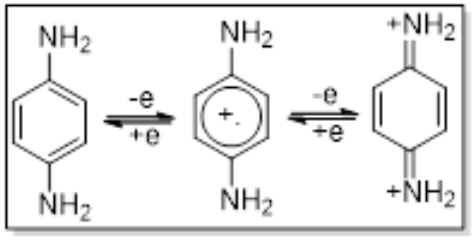


Figure 3: GC-MS Chromatogram of methanol extract of *F. bhotanica.*
1.7-Isobutoxy-5,9-dihydro-6,8-dioxa-7-bora-benzocycloheptene; 2.Dichloroaceticacid,dodecylester; 3. 1-Tetradecene; 4.1-Hexadecene; 5.2-Hexadecanol,
6.1,2-Benzenedicarboxylicacid,butyloctyl ester; 7.Benzenepropanoicacid,3,5-bis(1,1-dimethylethyl)-4-hydroxy,methylester; 8.Octatriacontylpentafluoropropionate.



Scheme 1

concentrations 50, 100, 200, 300µg/mL) of the tested plant extract to the above reaction mixture and recording the absorbance at 560nm. The % inhibition were expressed as in equation 1. By plotting the values of percentage inhibition as the abscissa and the concentrations as the ordinate, we calculated the IC_{50} value of all plant extracts in different solvents.

Electrochemical measurements of antioxidant activity by cyclic voltammetry

The measurement were done in DMF with TBAP as supporting electrolyte with scan rate 50 mV/sec. Pure nitrogen gas was passed through the solution before recording the voltammmogram.

Preparation of TBAP (tetra butyl ammonium perchlorate)

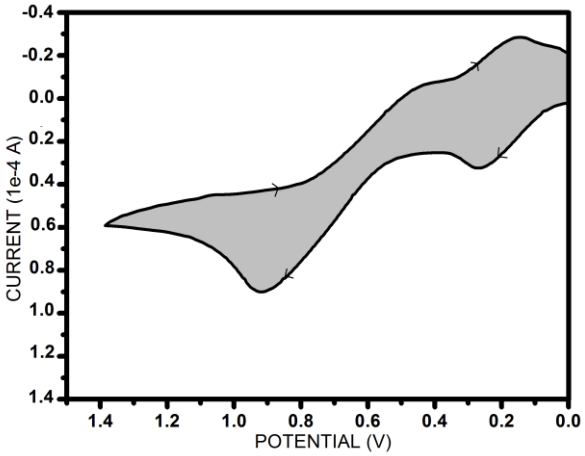


Figure 4.1: Cyclic voltammogram of 1,4-diaminobenzene

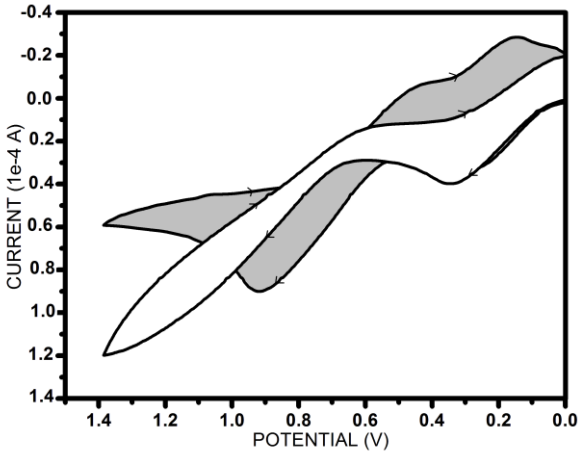


Fig.ure 4.2: Overlaid CV of 1, 4-diaminobenzene alone (shaded) and in presence of hexane extract of *F. bhotanica*(not shaded).

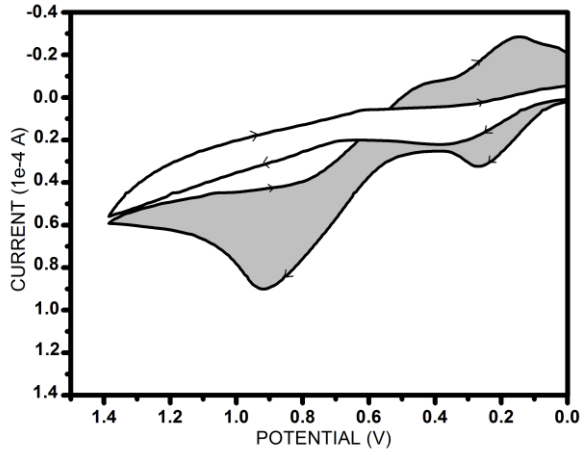


Figure 4.3: Overlaid CV of 1, 4-diaminobenzene alone (shaded) and in presence of ethylacetate extract of *F. bhotanica*(not shaded).

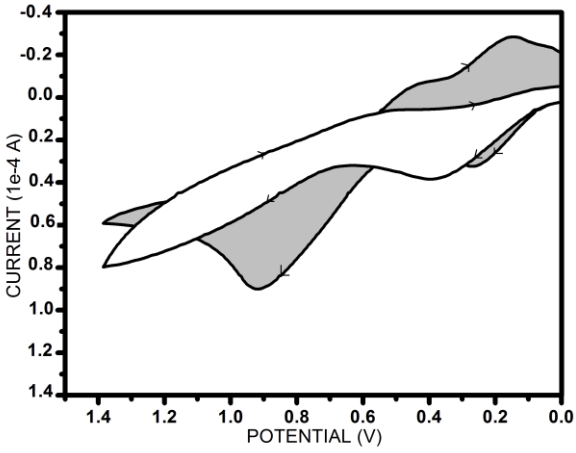


Figure 4.4: Overlaid CV of 1, 4-diaminobenzene alone (shaded) and in presence of methanol extract of *F. bhotanica*(not shaded).

Figure 4.1- 4.4: CV of 1.4-diaminobenzene alone (fig.4.1) and that in presence of hexane extract of *F. bhotanica* (fig.4.2), ethylacetate extract of *F. bhotanica* (fig.4.3) and methanol extract of *F. Bhotanica* (fig.4.4).

A saturated solution of 8.4 g of TBAB in 18 mL of water was treated with 2.1 mL of aqueous 70% $HClO_4$ to get insoluble perchlorate. The perchlorate so formed was filtered and washed with ice cold H_2O and dried. Recrystallization of the TBAP was done in cyclohexane-ethylacetate solution. To a saturated solution of TBAP in ethylacetate, cyclohexane was added to precipitate. Pure TBAP was dried at 100°C under vacuum.

Recording of Cyclic voltammorgam of 1, 4-diaminobenzene

The cyclic voltammogram of 1, 4-diaminobenzene was recorded by dissolving 4 mg of 1,4-diaminobenzene in DMF(3 mL) with 8 mg of TBAP as supporting electrolyte.

Recording of Cyclic voltammogram of 1,4-diaminobenzene in presence of the plant extracts

At first the cyclic voltammogram of 1,4-diaminobenzene was recorded as described above and to this solution 4 mg of the concentrated plant extract was added and mixed thoroughly. Then the cyclic voltammogram of the resulting solution was recorded as the same procedure. Pure nitrogen gas was passed through the solution before recording of each voltammogram. This experiment was done separately with each of the extracts, prepared to observe their effect on 1,4-diaminobenzene.

RESULTS AND DISCUSSION

Screening of Phytochemicals

The present study was carried out the hexane, ethyl acetate and methanol extract of *F. bhotanica*, which showed the presence of active phytochemical constituents. The phytochemical constituents found in the investigated plant extracts are summarized in Table 1. Alkaloid, saponins, flavonoids and phenolic compounds were present in the hexane, ethyl acetate and methanol extracts of leaves of *F. bhotanica*. It was found that tannins present only in ethyl acetate extract.

GC-MS analysis

GC-MS analysis is one of the best techniques to identify the constituents of volatile matter, long chain, branched hydrocarbons, alcohols, acids, ester etc. The GC-MS analysis of the hexane, ethyl acetate and methanol extract of leaves of *F. bhotinica* showed the presence of twenty

five, eleven and eight major compounds respectively that could contribute to the medicinal property of the plant. The identification of the phytochemical compounds was established on the basis of peak area, retention time and molecular formula. The compounds along with their retention time, molecular formula, molecular weight and peak area in percentage are presented in Table 2 for hexane extract, Table 3 for ethyl acetate extract and Table 4 for methanol extract. Their structure and biological activities are also shown in the tables. GC-MS chromatograms are presented in Fig.1 for hexane extract, Fig. 2 for ethyl acetate and Fig. 3 for methanol extract respectively.

It was observed that the compounds found in the hexane extract of *F. bhotanica* in the retention time from 16 min. to 19 min. are the cluster of dialkyl phthalates where the both alkyl groups may be same or different and the alkyl groups present are n-butyl, 2-methyl propyl, butyl iso hexyl, iso butyl-2-pentyl, butyl octyl, butyl-2-pentyl, 2-pentyl,butyl tetradecyl, 6-ethyl-3-octyl butyl, pentyl-2-pentyl and butyl-2-ethylhexyl. Some alkylpolysiloxan compounds were also found after the retention time at 22 min.

Among the compounds found in hexane extract and ethyl actetate extract, Dibutylphthalate was found with the highest peak area % (23.76) for hexane extract and % (39.97) for ethyl actetate extract. The compound 7-Isobutoxy-5,9-dihydro-6,8-dioxa-7-bora-benzocycloheptene was found with the highest peak area % (33.81) among the compounds in methanol extract.

It was observed that the four compounds, 2-hexadecanol; benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy, methyl ester; 1-tetradecene and 1-hexadecene were present in all the three solvent extracts*;* hexadecanoic acid, methyl ester and dibutylphthalate were common in hexane and ethyl acetate extract. The compound 1,2-benzenedicarboxylic acid butyl octyl ester were common in hexane and methanol extract.

Deoxy ribose assay

The hydroxyl radical scavenging ability of different solvent extracts of *F. bhotanica* was measured by using deoxy-ribose assay and studying competition between deoxyribose and plant extracts for hydroxyl radical generated from ferric-ascorbate-EDTA-H_2O_2 system. The formation of TBARS is decreased, when the plant extracts start to scavenge hydroxyl radicals. The % inhibition and IC_{50} value of hexane, ethylacetate and methanol extracts of *F. bhotanica* was shown in Table 5 and graphical presentation was shown in Fig. 4. It was observed that the methanol extract of has the highest hydroxyl radical scavenging ability compared to the other extracts with IC_{50} value 37.856±1.071µg/mL. The lowest hydroxyl radical scavenging ability was found in hexane extract with IC_{50} value 190.223 ± 0.547 µg/mL.

Xanthine oxidase inhibitory assay

The NBT superoxide radical scavenging assay relies on the competition between test substrate and nitro blue tetrazolium chloride (NBT) for superoxide anion generated by the xanthine/xanthine oxidase system. The decrease in absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. The results in Table 6 showed that of the methanol extracts of *F. bhotanica* has the highest superoxide anion radical scavenging potential in comparison with their n-hexane and ethyl acetate extracts. The IC_{50} value of methanol extract of *F. bhotanica* was found as 217.266 ±1.385 µg/mL.

Cyclic voltammetry

The effect of plant extracts of *F. bhotanica* on the electrochemical behaviour of 1,4-diaminobenzene has been studied with the help of cyclic voltammetry. 1,4-diaminobenzene has a well-defined redox cycle. Overall electrochemical process taking place is represented in scheme 1 where 1,4-diaminobenzyne can have benzene-benzenoid structure on electrochemical oxidation and reduction reaction. The well defined redox cycles of 1,4-diaminobenzene with $E_{1/2}$ at 208mV and $E_{1/2}$ at 674mV in DMF with the oxidation waves (Fig. 4.1) due to the formation of a radical cation and a diiminium dication respectively (Scheme 1). The first oxidation wave was observed at 264mV and the second oxidation wave was observed at 910mV. The first reversible cycle with $E_{1/2}$ at 208mV is due to formation of a cation radical, this radical in the second cycle with $E_{1/2}$ at 674mV transforms to a diimine.

The overall redox reactions of 1,4-diaminobenzene in presence of the plant samples have been significantly affected. The effects of different plant extracts of *F. bhotanica* are shown in Fig.4.1-4.3 and the shifts and/or absence of anodic potential of the oxidation waves of 1,4-diaminobenzene are summarized in Table 7. All the plant extracts of *F. bhotanica*, delayed the first oxidation wave. In all cases second oxidation was not observed, indicating the complete inhibition of the oxidation and demonstrated the potential radical scavenging effect of the extracts. Because once the cationic radical has been formed, due to radical scavenging ability of the extracts, the radical has become a non-radical and the second oxidation reaction was not possible.

CONCLUSION

The GC-MS analysis of hexane, ethyl acetate and methanol extract of *F. bhotanica* showed many biologically active compounds which have various uses. Some of the compounds have antibacterial, antimicrobial, antifungal, antioxidant, antimalarial, hypocholesterolemic, antiandrogenic, antifouling properties and some other compounds are used as nematicide, pesticide, surface active agent, lubricant additives etc.

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