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SCREENING AND EVALUATION OF BOTANICAL EXTRACT OF *PLECTRANTHUSFORSKOHLII* (WILLD) BRIQ.

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ABSTRACT

The present study was aimed to examine the phytochemical constituents present in the tuber of *P.forskohlii*. The preliminary phytochemical analysis confirmed the presence of various secondary metabolites like alkaloids, carbohydrates, tannins, phenolic, glycosides, amino acids, flavonoids and terpenoids. The GC-MS analysis determined the presence of seventy four different phytochemical compounds in methanolic extract of tuber of *P.forskohlii*. FT-IR results proved the presence of amides, aldehydes, alkene, alkyl halide, alcohol and aromatic compounds which shows major peaks at 3321.22, 2926.12, 1643.16, 1450.09, 1019.71, 578.74, 568.90 and 556.95 respectively. The results of the present study help to enhance the usage of *P.forskohlii* for its medicinal uses which is greatly depends on the diversified chemical substances present in the tuber of *P.forskohlii*.

Key words: *P.forskohlii*; Phytochemical; GC-MS analysis; FT-IR analysis.

INTRODUCTION

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. This knowledge is accessible from thousands of medical texts and manuscripts. The use of medicinal plants as traditional medicine is well known in rural areas of the many developing countries [1]. Herbs are mine of medicinal agents and large number of medicinal herbs is found to be efficacious, cheap and safe in preventing various diseases. Moreover, the use of herbal medicines for the treatment of different ailments is very important in developing countries where the cost of conventional medicines is a burden to the population. More than 30% of the entire plant species, at one time or other was used for medicinal purposes.

The genus *Plectranthus* (Lamiaceae) consists of some 350 species of perennial plants largely occurred in tropical Africa, Asia, Australia, the East India, the Malay Archipelago and the Philippines. Several species are grown as ornamental plants, leaf vegetables, root tuber vegetables [1, 2]. Because their economic, medicinal and other biological interest [3, 4]. *Plectranthus forskohlii*, has a rich diversity of ethnobotanical applications and is used as a condiment for heart ailments and stomach cramps [5]. It has been reported for its effect on intra-ocular pressure [6-8] and hypotensive [9, 10]. *P. forskohlii* has also been reported to have antianaphylactic [11], amoebicidal [12], antiplatelet [13, 14], gastroprotective [15], bronchodilating [16], anticancer [17] activity and prevents hair graying [18]. Forskolin is used as a research tool assessing the adenylatecyclase & cyclic AMP in cellular physiology [19].

Identification of individual components of complex mixtures such as terpenes/terpenoids in root hexane extracts requires the use of several techniques. GC-MS and FT-IR are useful tools in medicine and biological research aiming for the identification of mixtures and this method has already been applied successfully for the analysis of terpenoids, especially mono- and sesquiterpenes, in various plant extracts. Identification of the biomolecules found in an extracts by comparing their relative retention times/indices and their mass spectra. Therefore, the identified chemical constituents are used in folk medicine for a variety of diseases including infectious conditions. Other constituents of *P.forskohlii* like alkaloids, phenols and tannins have been reported to exhibit some biological activities like stimulating adenylcyclase, inhibition of platelet aggregation, mast cell degranulation, relaxation of the arteries, increasing the insulin secretion and thyroid function, decreasing adipose accumulation, reduction of body weight, treating skin diseases, cardiovascular disease, asthma, stimulating the secretion of digestive enzymes and absorption of nutrients in small intestine etc. [20-22].

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MATERIALS AND METHODS

Collection & Preparation of Plant Material

The tubers of *P.forskohlii* were collected from the natural habitats of Sirupakkam, Tamil Nadu, India. The tubers were washed three times thoroughly with running tap water to remove soil particles and adhered debris and finally with sterile distilled water. The tubers were cut, shade dried, ground into fine powder and stored in air tight container for further use.

Preliminary Phytochemical analysis

Shade dried and powdered plant material was successively extracted with Petroleum ether, Chloroform, Ethyl acetate and Methanol with gentle stirring for 72 hrs separately. The extracts were filtered with Whatman No1 filter paper and concentrated using vacuum distillation. The preliminary phytochemical constituents were analysed qualitatively by using standard method.

GC-MS analysis

10g of powdered sample is extracted with 30ml methanol overnight and filtered in ashess filter paper with sodium sulphate (2g) and the extract is concentrated to 1ml by bubbling nitrogen into the solution. The Clarus 500 GC used in the analysis employed a column packed with Elite-1(100% Dimethyl poly siloxane, 3nm X 0.25nm ID X 1um df) and the components were separated using helium (1ml/min) as the carrier gas. The 2ul sample extract injected into the instrument was detected by the Turbo mass gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 36th minute GC extraction process, the over was maintained at a temperature of 110°C with a 2min holding. The injector temperature was set at 25°C (Mass analyser). The different parameters involved in the operation of Clarus 500 MS, were also standardized (inlet line temperature: 200°C; Source temperature 200°C; Electron energy 70eV; Mass scan (m/2) 45-450). The MS detection was completed in 36 min. the relative percentage of each component was calculated by comparing its average peak to the total areas. The detection employed the NIST (National Institute of Standards and Technology) Version 2.0 year 2010 library. The compound prediction is based on Dr. Duke's Phytochemical and Ethno botanical databases by Dr. Jim Duke of the agricultural Research Science/USDA.

FT-IR Analysis

The FT-IR analysis was performed using Perkin Elmer Spectrum Version 10.03.09 system, which was used to detect the functional groups of the compound. A small amount of compound was placed directly on the zinc solenoid piece and constant pressure. Data of infrared absorbent, collected over the wave number ranged from 3500 cm-1 to 500 cm-1 using spectra software. Samples were run in triplicate and all of them were undertaken within a day period.

RESULTS AND DISCUSSION

The preliminary phytochemical screening was performed for the plant material and the study revealed that Alkaloids, carbohydrates, glycosides, amino acids, phenolic, flavonoids, tannins and terpenoids were reported in Table 1. The phytochemical constituents present in the tuber of *P.forskohlii* were reported in Table 2. GC-MS analysis of methanolic extract revealed the presence of 74 compounds. Out of this 74 compound 12 compounds were majorly present in the analysis namely 8,13, Epoxy Labdan-1,6,7,9-tetraol-11-one, Decanal dimethyl acetal, Spiro (furan-2(5H),2'(1'H)-naptho (2,1-b) furan)-5-one, E,E,Z-1,3,12-Nanodecatriene-5, Spiro(4,5) decan-7-one, Acetic acid, decyl ester, n-Hexadecanoic acid, Borinic acid, ABIETA-9 (11), 8 (14),12-TRIEN-12-OL, Cembra-2,7,11-trien-4,5-diol, 6-Beta-Hydroxy 6-alpha-pentyl 3-oxa-a-homo-5, 9,12-Octadecanoic acid(Z,Z). The GC-MS spectrum confirmed the presence of 12 major components with the retention time 23.088, 8.401, 19.569, 17.179, 21.068, 8.866, 15,279, 10.269, 18.866, 24.185, 20.837 and 16.945. Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute of Standards and Technology (NIST08s) and WILEY8 and FAME having more patterns. The spectrum of unknown component was compared with the spectrum of known components stored in the NIST08s, WILEY8 and FAME library. The name, molecular formula, molecular weight and structure of the component of the test material were determined (Fig 1). The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The methanolic extract of *P.forskohlii* tubers FT-IR analysis results proved the presence of amides, aldehydes, alkene, alkyl halide, alcohol and aromatic compounds which shows major peaks at 3321.22, 2926.12, 1643.16, 1450.09, 1019.71, 578.74, 568.90 and 556.95 respectively (Fig 2 & Table 3).

Table 1. Preliminary Phytochemical analysis of tuber of *P.forskohlii* (Willd) Briq

Phytochemicals	PE	CH	EA	E	M
Alkaloids	-	-	+	+	+
Carbohydrates	-	-	+	+	+
Saponins	+	+	+	-	-
Glycosides	-	-	+	+	+
Amino acids	-	-	-	+	+
Phytosterol	+	-	-	-	-
Phenolic compounds	-	+	+	+	+
Flavanoids	-	-	+	+	+
Terpinoids	-	-		+	+
Tannins	-	-	+	+	+

PE – Petroleum ether, CH – Chloroform, EA – Ethyl acetate, E – Ethanol, M – Methanol extracts; (+) Positive, (-) Negative.

Table 2. Phytoconstituents present in the Methanolic tuber extract of *P.forskohlii* (Willd) Briq.Using GC-MS

Peak	R. Time	% of Area	Formula	M.Wt	Name of the Compounds
1	5.341	0.12	$C_9H_{20}O$	144	1-Nonanol
2	5.443	0.21	$C_{14}H_{17}F_7O_2$	350	Borneol
3	5.895	2.80	$C_{10}H_{20}O$	156	Decanal
4	6.636	0.12	$C_{17}H_{23}NO_2$	273	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate
5	6.891	1.90	$C_{10}H_{22}O$	158	1-Decanol
6	7.210	2.45	$C_{12}H_{20}O_2$	196	Bornyl acetate
7	8.401	7.42	$C_{12}H_{26}O_2$	202	Decanal dimethyl acetal
8	8.866	4.45	$C_{12}H_{24}O_2$	200	Acetic Acid, Decyl Ester
9	9.214	0.26	$C_{15}H_{24}$	204	2,6,10,10-tetra methyl bicyclo
10	9.533	0.05	$C_{15}H_{24}$	204	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)
11	9.942	0.16	$C_9H_{10}O_2$	150	Benzoic acid, 2,5-dimethyl
12	10.062	0.10	$C_{15}H_{24}$	204	(-)-Sinularene
13	10.269	3.57	$C_8H_{20}B_{20}$	154	Borinic acid, diethyl-, anhydride
14	10.490	1.00	$C_{15}H_{24}$	204	Trans(.beta.)-Caryophyllene
15	10.891	0.13	$C_{12}H_{24}O_2$	200	Dodecanoic acid
16	11.007	0.36	$C_{14}H_{30}O_2$	230	Dodecanal Dimethylacetal
17	11.398	0.14	$C_{20}H_{30}O_5$	350	Andrographolide
18	11.631	0.22	$C_{15}H_{26}O$	222	Cedrol
19	12.213	0.26	$C_{12}H_{20}$	164	1H-Indene, 1-ethylideneoctahydro-7a-methyl-, cis
20	13.177	0.14	$C_{18}H_{34}O_2$	282	9-Octadecenoic Acid (Z)
21	14.883	0.07	$C_{21}H_{42}O_2$	326	Eicosanoic Acid, Methyl Ester
22	15.279	3.60	$C_{16}H_{32}O_2$	256	n-Hexadecanoic acid
23	15.850	0.43	$C_{20}H_{34}O$	290	1H-Naphtho[2,1-b]pyran, 3-ethenyldodecahydro-3,4a,7,7,10a
24	16.231	0.09	$C_{18}H_{34}O$	266	9-Octadecenal, (Z)
25	16.438	1.99	$C_{20}H_{30}$	270	7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophe
26	16.817	0.10	$C_{11}H_{19}C_{10}$	202	10-undecenoyl chloride
27	16.945	3.05	$C_{18}H_{32}O_2$	280	9,12-Octadecadienoic acid (Z,Z)
28	17.179	7.28	$C_{19}H_{34}O_2$	294	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
29	17.337	0.53	$C_{19}H_{34}O_2$	294	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
30	17.450	1.77	$C_{30}H_{52}O_2$	444	Tetracosa-2,6,14,18,22-pentaene-10,11-diol, 2,6,10,15,19,23
31	17.576	0.22	$C_{15}H_{26}O_2$	238	Widdrolhydroxyether
32	17.684	0.24	$C_{32}H_{52}O_2$	468	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.b
33	18.046	0.10	$C_{15}H_{24}O_2$	236	Spiro (4,5) decan-7-one
34	18.142	0.17	$C_{27}H_{48}$	372	Cholestane
35	18.206	1.33	$C_{13}H_{24}O$	196	3aH-Inden-3A-OL, Octahydro-1,4,4,7A-Tetramethyl-,
36	18.390	1.04	$C_{16}H_{28}O_2$	252	Sclaral
37	18.492	0.28	$C_{24}H_{40}O_4$	392	Deoxycholic acid
38	18.543	0.26	$C_{15}H_{24}O_2$	236	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-
39	18.677	0.86	$C_{16}H_{26}O_3$	266	2,5-Furandione,3-(Dodecynyl) Dihydro
40	18.866	3.57	$C_{20}H_{30}O$	286	Abieta-9(11),8(14),12-Trien-12-OL #
41	19.009	0.84	$C_{19}H_{36}$	264	1H-Indene, 5-butyl-6-hexyloctahydro-
42	19.164	1.64	$C_{15}H_{26}O$	222	1H-Benzocyclohepten-7-OL, 2,3,4,4A,5,6,7,8-Octahydro-
43	19.256	0.13	$C_{14}H_{16}O_5$	232	1H-Thioxanthene-4-carboxaldehyde, 2,3,5,6,7,8-

44	19.361	1.08	$C_{27}H_{30}O_{15}$	594	03027205002 Flavone 4'-OH,5-OH,7-DI-O-Glucoside
45	19.569	7.27	$C_{20}H_{30}O_3$	318	Spiro[furan-2(5H),2'(1'H)-naphtho[2,1-b]furan]-5-one, 3'a,4',5
46	19.697	0.76	$C_{20}H_{28}O_2$	300	9(1H)-Phenanthrenone, 2,3,4,4a,10,10a-hexahydro-6-hydroxy-
47	19.774	0.21	$C_{13}H_{22}O$	194	1a,2,5,5Tetramethyl-trans-1a,4a,5,6,7,8-hexahydro-gamma-chr
48	19.863	1.22	$C_{20}H_{26}O_2$	298	Podocarpa-1,8,11,13-Tetraen-3-One, 12-Hydroxy
49	20.003	0.14	$C_{20}H_{28}O_2$	300	9(1H)-Phenanthrenone, 2,3,4,4A,10,10A-Hexahydr
50	20.118	0.48	$C_{21}H_{23}IO_7$	514	ent-3a-Acetoxy-10-hydroxy-13-iodomethyl-16-oxo-8,13-epi-17
51	20.323	0.32	$C_{20}H_{28}O_2$	300	9(1H)-Phenanthrenone, 2,3,4,4A,10,10A-Hexahydr
52	20.432	0.89	$C_{28}H_{40}O_{10}$	536	4a,7a-Epoxy-5H-cyclopenta[a]cyclopropa[f]cycloundecene-2,4
53	20.621	0.35	$C_{16}H_{26}O_3$	266	2-Dodecen-1-yl(-)succinic anhydride
54	20.752	0.29	$C_{18}H_{21}NO_5$	331	1,4A-Dimethyl-7-Nitro-9-Oxo-1,2,3,4,4A,9,10,10A-O
55	20.837	3.34	$C_{24}H_{38}O_4$	390	6.beta.-Hydroxy-6.alpha.-pentyl-3-oxa-a-homo-5.alpha.-andros
56	21.068	5.99	$C_{15}H_{24}O_2$	236	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl
57	21.185	0.15			RT:21.183
58	21.279	0.83	$C_{20}H_{34}O_2$	306	(1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien-4,5-diol
59	21.540	0.39	$C_{24}H_{36}O_5$	404	7,12-diketolithocholic acid
60	21.669	0.08	$C_{32}H_{42}O_{10}$	586	D-Homo-24-nor-17-oxachola
61	21.805	0.93	$C_{15}H_{24}O_2$	236	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl
62	21.886	0.16	$C_{26}H_{36}O_9$	492	Ingol-3,8,12-Triacetat
63	22.084	0.57	$C_{30}H_{60}O_2$	452	Hexadecanoic acid, tetradecyl ester
64	22.275	0.32	$C_{15}H_{24}O_5$	284	Dihydroqinghaosu
65	22.475	0.61	$C_{20}H_{34}O_2$	306	(1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien-4,5-diol
66	22.700	0.95	$C_{20}H_{34}O_2$	306	(1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien-4,5-diol
67	23.088	11.31	$C_{22}H_{32}O_7$	408	8,13-Epoxy-labadan-1,6,7,9-tetraol-11-one, 7-O-acetate(ester)
68	23.918	0.66	$C_{19}H_{34}O_2$	294	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
69	24.185	3.44	$C_{20}H_{34}O_2$	306	(1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien-4,5-diol
70	25.197	0.08	$C_{32}H_{42}O_{10}$	586	D-Homo-24-nor-17-oxachola
71	28.922	0.32	$C_{28}H_{48}O$	400	Ergost-5-en-3-ol, (3.Beta.,24R)
72	29.584	0.82	$C_{29}H_{48}O$	412	Stigmasterol
73	30.926	0.32	$C_{29}H_{50}O$	414	Stigmast-5-EN-3-ol, (3.Beta.)-
74	32.910	0.27	$C_{29}H_{46}O$	410	4,22-Stigmastadiene-3-one

Table 3. FT-IR absorption and functional group of tuber of *P.forskohlii* (Willd) Briq.

S No	Wave No	Molecular Motion	Functional group	Absorption intensity
1	3321.22	N-H stretch	Amides	Medium
2	2926.12	C-H stretch	Aldehydes	Weak
3	1643.16	C=C stretch	Alkenes	Medium
4	1450.09	C-C stretch	Aromatic	Medium
5	1019.71	C-O stretch	Alcohol	Strong
6	578.74	C-Br stretch	Alkyl halide	Strong
7	568.90	C-Br stretch	Alkyl halide	Strong
8	556.95	C-Br stretch	Alkyl halide	Strong

Fig 1. GC-MS chromatogram of methanolic tuber extract of *P.forskohlii* (Willd) Briq

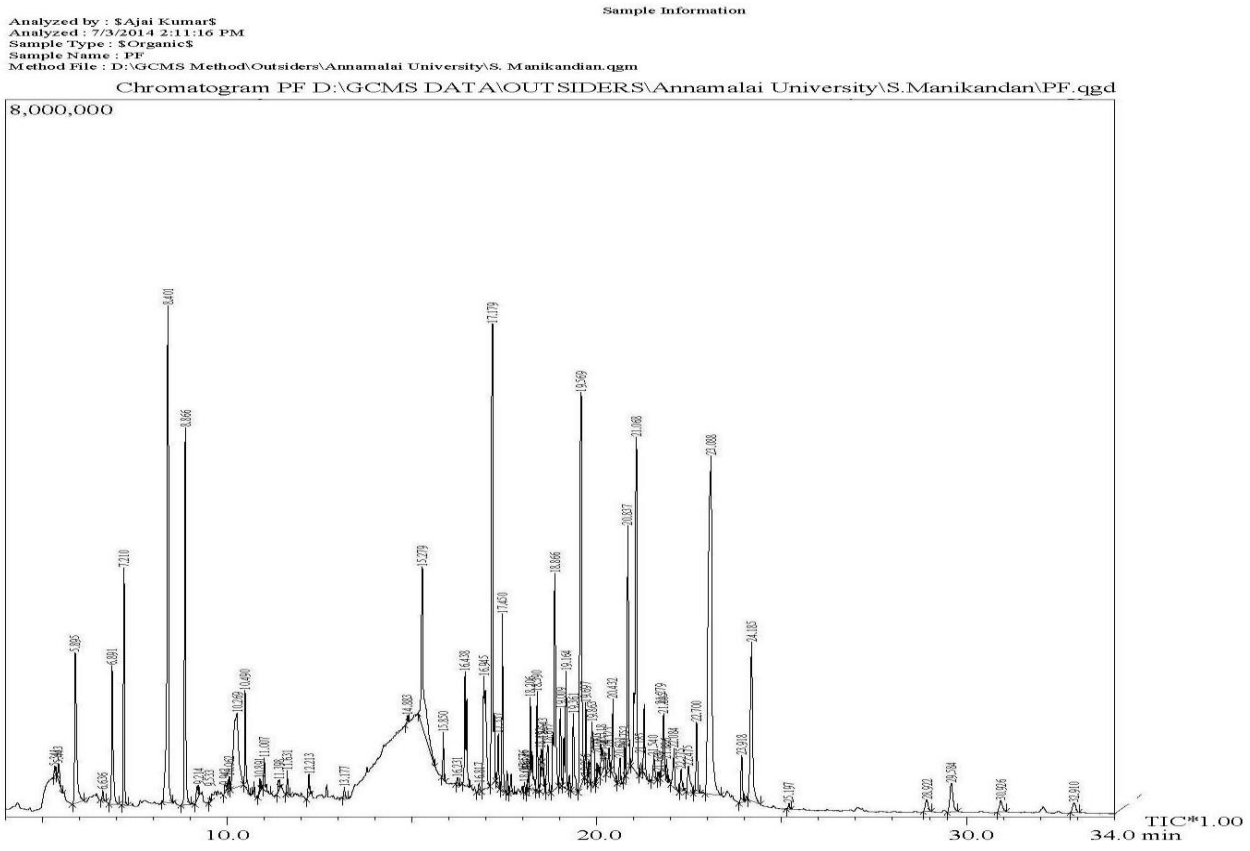
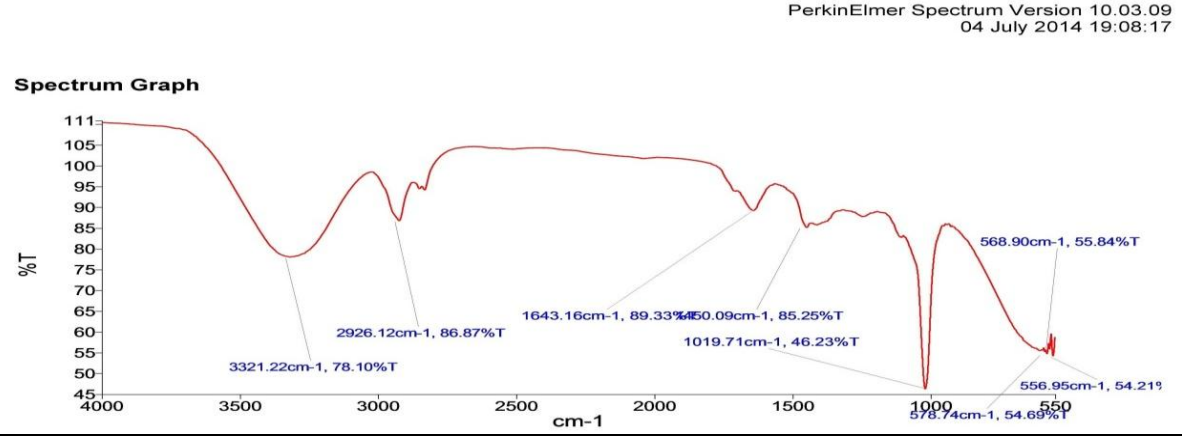


Fig 2. FT-IR Spectrum of methanolic tuber extract of *P.forskohlii* (Willd) Briq



CONCLUSION

In the present study, we observed 74 compounds from methanolic extract of tuber of *P.forskohlii*. The result of the present study conform traditional applications of the medicinal plant *P.forskohlii*. The tuber of *P.forskohlii* can be used as anti-obesity, hypertension, asthma, bronchial diseases, heart disorder, glaucoma, psoriasis, antithrombotic effect, depression, increasing lean body mass, anticancer, anti-diabetics, skin diseases, cardiovascular disease and breathing problems etc., the present study enhances the traditional usage of *P.forskohlii* which possess several known and unknown bioactive compounds, new drug can be formulated to treat various diseases. By using FT-IR spectrum, we can conform the functional constituents from given extract, identify the medicinal materials from the adulterate and even evaluate the quantities of medicinal materials. Many researchers applied the FT-IR spectrum as tool for distinguish closely associated plants and other organisms. The results of the present study developed novel phytochemical marker to identify the medicinally important plant. Further advanced spectroscopic studies are required for the structural elucidation and identification of active principles present in the tuber of *P. forskohlii*.

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