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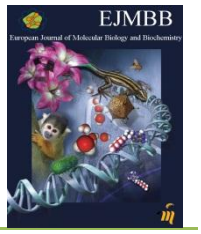

PHYTOCHEMICAL STUDIES ON *POLYGONUM GLABRUM* WILLD

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ABSTRACT

The present study was aimed to explore the biochemical and phytochemical profile of *Polygonum glabrum* (Willd.). To identify the presence or absence of metabolites like lignin, tannin, chitin, suberin, phenols and poly phenols in the leaves, stem and root of *P. glabrum* histochemical analysis were performed. Preliminary qualitative phytochemical screening of *P. glabrum* leaves extract was carried out. To reveal the functional metabolites and elements UV-Vis, FT-IR and Energy Dispensive X-ray Spectrometer analysis were performed. The occurrence and distribution of various metabolites viz., lignins, polyphenols and tannins on various parts of *P. glabrum* were illustrated. The epidermis and vascular bundles of root and stem showed higher amount of metabolites than cortex. Preliminary phytochemical analysis revealed the presence of steroids, alkaloids, phenolic groups, cardiac glycosides, flavonoids, saponins, tannins and sterols in *P. glabrum*. The extracts of *P. glabrum* demonstrated varied spectrum with different absorption and the results of UV-Vis analysis were tabulated. The results of *P. glabrum* FTIR analysis confirmed the presence of alkynes, alkenes, esters, ethers, aromatics, alkyl halides, aliphatic amines, carboxylic acids, aldehydes, nitro compounds, primary, secondary, tertiary amines and phenols. The EDS analysis revealed the occurrence of four elements viz., carbon, oxygen, chlorine and potassium in the ethanolic extract of *P.glabrum.* The results of phytochemical and biological analysis showed that the metabolites present in the leaves of *P. glabrum* can be used to cure various diseases.These spectroscopic profiles will act as pharmacognostic marker to distinguish the medicinally important *P. glabrum* using relatively simple, cost-effective spectroscopic profile from its adulterants in the pharmaceutical industries.

INTRODUCTION

Polygonum glabrum Willd is an erect annual herbaceous plant particularly found in river banks, stream sides and marshy areas have been used as folk medicine and as ingredient in various Ayurvedic preparations. Traditionally rootstock of *P. glabrum* used in pneumonia, consumption, jaundice, fevers and leaves are used as antispasmodic, astringent, diuretic, rubefacient and vermifuge. A decoction of the plant has been used as a foot and leg soak in the treatment of rheumatism [1]. *P. glabrum* extract contains some pharmacologically active principles and possess antioxidant properties and hepatoprotective activity [2,3], analgesic activity [4], anti inflammatory [5] and antidepressant properties. Drug discovery from medicinal plants involve a multifaceted approach combining botanical, phytochemical, biological and molecular techniques. The spectrometric and chromatographic screening methods could provide the needed preliminary observations to select crude plant extracts with useful properties for further chemical and pharmacological studies [6]. Spectroscopic (UV-Vis, FTIR) methods together or separate can be used in predictable methods [7]. The present need for element analysis in biochemistry, bioinorganic and clinical chemistry is to justify the present demand for innovative chemical speciation strategies and analytical technologies.

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With this knowledge, the present study was aimed to explore the biochemical and phytochemical profile of the medicinally important *Polygonum glabrum* (Willd.).

MATERIALS AND METHODS

The mature and healthy plant *Polygonum glabrum* Willd was collected from Cheranmahadevi, Tirunelveli district, Tamil Nadu, India in November 2013. The leaves were isolated from the mother plants and dried in shade for two weeks. The shade dried leaves were ground using mechanical grinder. The powder of *Polygonum glabrum* (10g) was sequentially extracted with petroleum ether, chloroform and ethanol using soxhlet apparatus for 8 h. For aqueous extracts the dried powder were boiled with water for 24 h continuously. The supernatants were filtered and make up to known volume for phytochemical studies. Histochemical tests were made on the fresh sections of the plant treated with the various reagents to identify the presence or absence of metabolites like lignin, tannin, chitin, suberin, phenols and poly phenols in the leaves, stem and roots of *P. glabrum* [8-11]. Preliminary qualitative phytochemical screening of *P. glabrum* leaves extract was carried out by following standard phytochemical method described by Harborne [12].

Fluorescence analysis

To reveal the fluorescence characters of *P. glabrum*, leaves extracts of *P. glabrum* were examined under visible and UV light by the standard method [13]. The characteristic of colour reaction were compared with a standard colour chart and the observations were tabulated.

Spectroscopic analysis

For UV-Vis and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-1100 nm using Shimazdu Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the UV-Vis and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

EDS analysis

To identify the weight percentage of major and minor elements present in the leaves of *P. glabrum* EDS analysis was carried out using OXFORD INCA Energy Dispensive X-ray Spectrometer (EDS).

RESULTS

Histochemical studies

The occurrence and distribution of various metabolites viz., lignins, polyphenols and tannins on various parts of *P. glabrum* were illustrated in Table 1; Fig 1. The epidermis and vascular bundles of root and stem showed higher amount of metabolites than cortex.

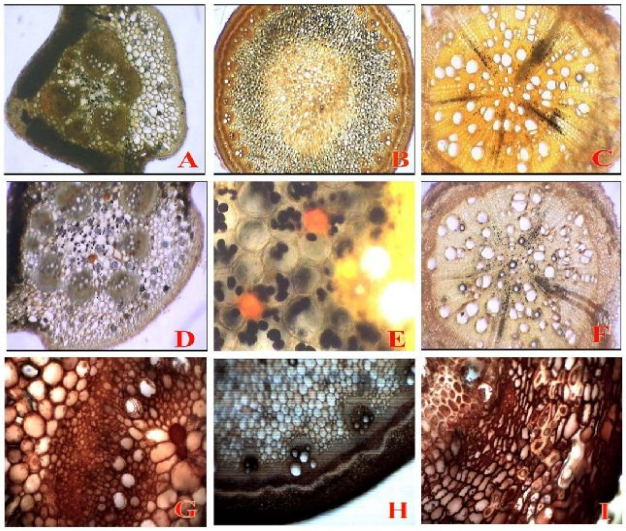


Fig 1: Histochemical studies of *Polygonum glabrum* Willd.

A,D,G: Presence of lignin, tannin and polyphenol in *Polygonum glabrum* leaf; B,E,H: Presence of lignin, tannin and polyphenol in *Polygonum glabrum* stem; C,F,I: Presence of lignin, tannin and polyphenol in *Polygonum glabrum* root.

Phytochemical analysis

Thus out of 50 tests for the presence or absence of compounds, 25 tests revealed the presence of steroids, alkaloids, phenolic groups, cardiac glycosides, flavonoids, saponins, tannins and sterols in *P. glabrum* (Table 2). Chloroform, acetone and ethanolic extracts of *P. glabrum* showed the maximum presence (60% 6 out of 10) other than tested extracts. Petroleum ether extracts of *P. glabrum* demonstrated minimum presence (30%). Steroids showed its presence in all the extracts except aqueous extract of *P. glabrum*. Alkaloids confirmed its presence only in the chloroform extracts of *P. glabrum.* Phenolic groups conferred its existence in acetone, ethanolic, chloroform and aqueous extracts of *P. glabrum*. Cardiac glycosides determined its occurrence in all the five extract. Flavonoids illustrated its existence in ethanolic and aqueous extracts of *P. glabrum.* Saponins failed to show its presence in any of the tested extracts of *P. glabrum.*

Fluorescence analysis

In drug discovery the fluorescence analysis is an important pharmacognostic parameter that will reveal the nature of chromopores present in a drug source. The nature of drug / powder fluorescence characters serves as biomarker to identify the similar drugs and distinguish its adulterants. The result fluorescence analysis is demonstrated with varied colours under visible and UV light (Table 3).

UV- Vis Analysis

With reference to solvents, the extracts of *P. glabrum* demonstrated varied spectrum with different

absorption and the results of UV- Vis analysis were tabulated in Table 4. The petroleum ether extract of *P. glabrum* showed the peaks at 660, 608, 558, 535 and 445 nm with the absorption 3.068, 1.238, 0.596 and 1.539 respectively. The chloroform extract of *P. glabrum* illustrated active peaks at 656, 608 and 538 nm. The acetone extract of *P. glabrum* demonstrated peaks at 891, 655, 607, 557 and 534nm with the absorption of 0.117, 3.215, 1.969, 1.263 and 2.498 correspondingly. The ethanolic extract of *P. glabrum* displayed the peak occurrence at 664, 606, 537 and 321nm. The aqueous extract of *P. glabrum* showed single peak detection at 355 nm.

FTIR analysis

The results of *P. glabrum* FTIR analysis confirmed the presence of alkynes, alkenes, esters, ethers, aromatics, alkyl halides, aliphatic amines, carboxylic acids, aldehydes, nitro compounds, primary, secondary, tertiary amines and phenols with different peak values ranging from 516.92 to 3442.74 in the tested extracts of *P. glabrum* (Table 5; Fig. 2-6). The FTIR analysis results confirmed the functional group variation in the tested extracts. The variation in the tested extracts of *P. glabrum* may be due to the solvents solubility.

EDS analysis

The EDS analysis revealed the occurrence of four elements viz., carbon, oxygen, chlorine and potassium in the ethanolic extract of *P.glabrum* (Table 6).

Table 1. Histochemical Studies on *P.glabrum*

Metabolites	Root			Leaves			Stem		
	Epidermis	Cortex	VB	Epidermis	Cortex	VB	Epidermis	Cortex	VB
Lignin	+++	++	+++	+	+++	++	+++	++	+++
Tannin	+++	+++	+++	++	++	+++	+++	++	+++
Poly Phenol	+++	+++	+++	+++	+++	+++	+++	++	+++

Table 2. Preliminary phytochemical analysis of *P. glabrum*

Compounds	Petroleum ether	Chloroform	Acetone	Ethanol	Aqueous
Steroids	+	+	+	+	-
Alkaloids	-	+	-	-	-
Phenolic groups	-	+	+	+	+
Cardiac glycosides	+	+	+	+	+
Flavonoids	-	-	-	+	+
Saponins	-	-	-	-	-
Tannins	-	-	+	+	+
Caumarine glycosides	-	+	-	+	-
Terpenoids	-	-	+	-	-
Sterols	+	+	+	-	-

Table 3. Fluorescence analysis of *P. glabrum*

Solvents	Visible light	UV light
Petroleum ether	green	green
Chloroform	dark green	dark green
Acetone	green	green
Ethanol	red	green
Aqueous	Brown	dark green
50% HNO_3	light green	green
1N HCl	Yellow	light green
1N NaOH	green	dark green
1N H_2SO_4	Orange	red

Table 4. UV- Visible spectrum of *P. glabrum*

Petroleum ether		Chloroform		Acetone		Ethanol		Aqueous	
nm	Abs	nm	Abs	nm	Abs	nm	Abs	nm	Abs
660	3.068	656	3.135	891	0.117	664	2.201	355	3.718
608	1.238	608	1.751	655	3.215	606	0.625		
558	0.596	538	2.135	607	1.969	537	0.767		
535	1.539			557	1.263	321	4		
445	4			534	2.498				

Table 5. FT-IR peak values with functional groups of *P.glabrum*

Peak	Functional group	P	C	A	E	Aq
516.92	alkyl halides (c-Br stretch)	-	-	-	+	-
570.93	alkyl halides (c-Br stretch)	-	-	-	-	+
594.08	alkyl halides (c-Br stretch)	-	-	+	-	-
624.94	alkyl halides, alkynes (c-Br stretch), –C(triple bond)C–H: C–H bend	-	-	-	+	-
655.8	alkyl halides,alkynes,alkenes, (–C(triplebond)C–H: C–H bend), (c-Br stretch), (=C–H bend)	-	-	-	-	+
686.66	alkyl halides,alkynes,alkenes, aromatics (–C(triple bond)C–H: C–H bend), (c-Br stretch), (=C–H bend)	-	-	+	-	-
725.23	alkanes, alkenes,aromatics, primary, secondary amines (N–H wag), (C–H rock), (C–H " oop")	+	+	-	-	-
740.67	alkylhalides, alkenes, aromatics, primary, secondary amines (N–H wag), (C–H rock), (C–H " oop")	-	-	-	-	+
763.81	alkylhalides, alkenes, aromatics, primary, secondary amines (N–H wag), (C–H rock), (C–H " oop")	-	-	+	+	-
810.1	alkylhalides, alkenes, aromatics, primary, secondary amines (N–H wag), (C–H rock), (C–H " oop")	-	-	+	-	-
825.53	alkylhalides, alkenes, aromatics, primary, secondary amines (N–H wag), (C–H rock), (C–H " oop")	-	-	-	+	-
840.96	alkylhalides, alkenes, aromatics, primary, secondary amines (N–H wag), (C–H rock), (C–H " oop")	-	-	+	-	+
848.68	alkylhalides, alkenes, aromatics, primary, secondary amines (N–H wag), (C–H rock), (C–H " oop")	+	+	-	-	-
871.82	alkenes, aromatics, primary, secondary amines (=C–H bend), (C–H " oop"),(N–H wag)	-	-	-	+	-
918.12	alkenes, carboxylic acids, (O–H bend), (=C–H bend)	+	-	-	-	-
972.12	alkenes (=C–H bend)	+	-	+	-	+
995.27	alkenes (=C–H bend)	-	-	+	-	+
1041.56	aliphatic amines, alcohols, carboxylic acids, esters, ethers (C–O stretch), (C–N stretch)	-	-	-	+	-
1064.71	aliphatic amines, alcohols, carboxylic acids, esters, ethers. (C–O stretch), (C–N stretch)	-	-	+	-	+
1067.71	aliphatic amines, alcohols, carboxylic acids, esters, ethers. (C–O stretch), (C–N stretch)	-	-	-	-	+
1087.85	aliphatic amines, alcohols, carboxylic acids, esters, ethers. (C–O stretch), (C–N stretch)	+	-	-	-	-
1103.28	aliphatic amines, alcohols, carboxylic acids, esters, ethers. (C–O stretch), (C–N stretch)	-	-	-	+	-
1118.71	aliphatic amines, alcohols,carboxylic acids, esters, ethers (C–Ostretch)	-	-	-	-	+
1157.29	alcohols, carboxylic acids, esters, ethers,alkyl halides, aliphatic amines (C–O stretch), (C–N stretch)	+	-	-	-	-
1165	alcohols, carboxylic acids, esters, ethers,alkyl halides, aliphatic amines (C–O stretch), (C–N stretch)	-	+	+	-	+
1226.73	alcohols, carboxylic acids, esters, ethers,alkyl halides, aliphatic amines (C–O stretch), (C–N stretch)	-	-	-	+	-
1234.44	alcohols, carboxylic acids, esters, ethers,alkyl halides, aliphatic amines (C–O stretch), (C–N stretch)	+	-	-	-	-
1249.87	alcohols, carboxylic acids, esters, ethers,alkyl halides, aliphatic amines (C–O stretch), (C–N stretch)	-	-	+	-	+
1257.59	alcohols, carboxylic acids, esters, ethers,alkyl halides, aliphatic amines, aromatic amine (C–N stretch)	-	+	-	-	-
1350.17	nitro compounds, alkanes, (N–O symmetricstretch), (C–H rock)	-	-	-	+	-
1442.75	aromatics (C–H " oop"),	-	-	-	+	-
1458.18	alkanes, aromatics (C–H " oop"), (C–H bend)	+	+	+	-	+
1527.62	nitro compounds (N–O symmetricstretch)	-	-	-	+	-
1543.05	nitrocompounds (N–O symmetricstretch)	-	-	+	-	-
1620.21	primary amines (N–H bend)	-	-	-	+	-
1627.92	primary amines (N–H bend)	-	-	+	-	-
1635.64	primary amines (N–H bend)	-	+	-	-	-

1651.07	alkenes (–C=C– stretch)	+	-	-	-	-
1705.07	carbonyl (general), carboxylic acids, alba, beta - unsaturated aldehydes,ketones (C=O stretch)	-	-	-	+	-
1735.93	carbonyl (general), carboxylic acids, esters,saturatedaliphatic,aldehydes, saturatedaliphatic (C=O stretch)	+	+	+	-	+
2175.7	alkynes (–C(triplebon d)C– stretch)	-	-	+	-	+
2584.61	carboxylic acids (O–H stretch)	-	-	-	-	+
2723.49	aldehydes,carboxylic acid (O–H stretch), (H–C=O: C–H stretch)	-	-	+	-	+
2839.22	carboxylic acids (O–H stretch)	-	-	+	-	+
2854.65	carboxylic acids (O–H stretch)	+	+	-	+	-
2877.79	carboxylic acids (O–H stretch)	-	-	-	-	+
2924.09	carboxylic acids,alkanes (O–H stretch), (C–H stretch)	+	+	-	+	-
2939.52	carboxylic acids,alkanes (O–H stretch), (C–H stretch)	-	-	+	-	-
2947.23	carboxylic acids,alkanes (O–H stretch), (C–H stretch)	-	-	-	-	+
2970.38	carboxylic acids,alkanes (O–H stretch), (C–H stretch)	-	-	+	-	-
3194.12	carboxylic acids (O–H stretch)	-	-	+	-	+
3371.57	alcohols, phenols, primary, secondary amines, amides (O–H stretch , H–bonded),(N–H stretch)	-	-	-	+	-
3402.43	alcohols, phenols (N–H stretch)	+	-	-	-	-
3448.72	alcohols, phenols (N–H stretch)	-	-	+	-	+

P – Petroleum ether; C – Chloroform; A - Acetone; M -methanol; Aq – Aqueous.

Table 6. Concentration of macro and micro elements in *Polygonum glabrum* through EDS analysis

Element	Weight %	Atomic %
C	69.32	75.70
O	28.88	23.67
Cl	0.73	0.27
K	1.07	0.36

Fig. 2. FT-IR Spectrum of *P. glabrum* Petroleum ether extracts

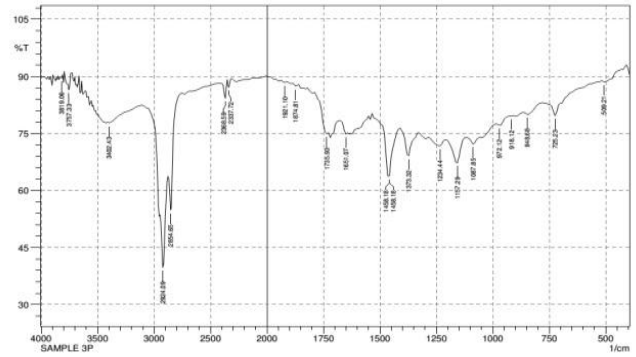


Fig. 3. FT-IR Spectrum of *P. glabrum* Chloroform extracts

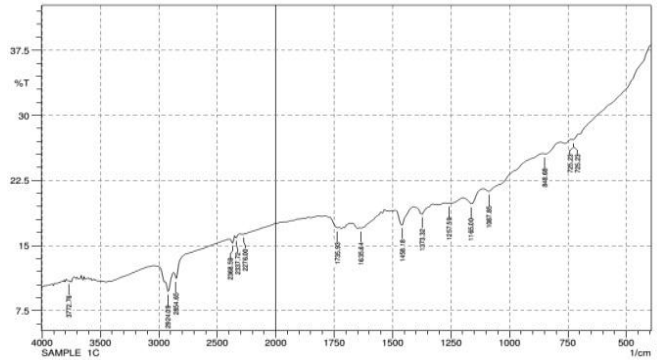


Fig. 4. FT-IR Spectrum of *P. glabrum* Acetone extracts

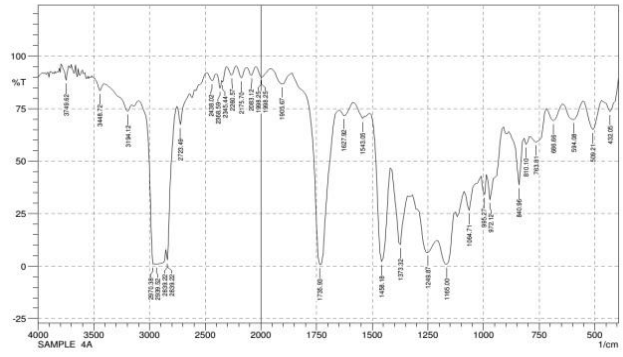


Fig. 5. FT-IR Spectrum of Methanolic extracts

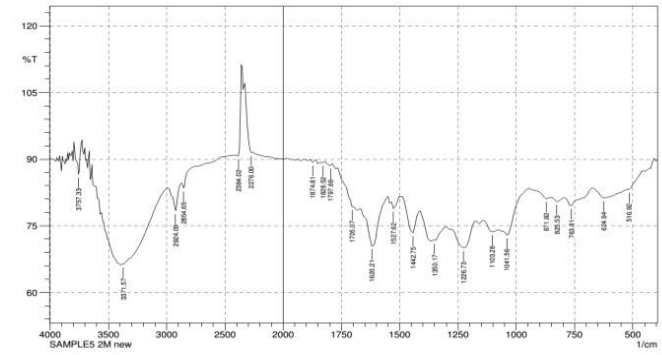


Fig. 6. FT-IR Spectrum of Aqueous extracts

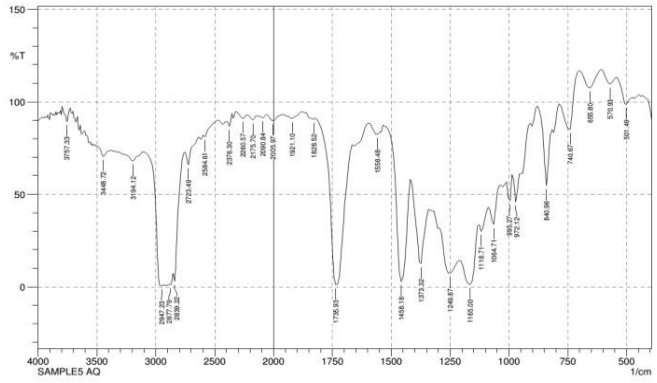
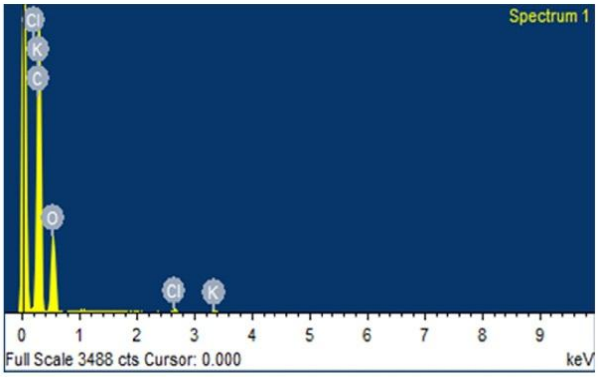


Fig 7. EDS Spectrum of *Polygonum glabrum*



DISCUSSION

Current medicinal research covers all the latest and outstanding developments in medicinal chemistry and rational drug design. Each issue contains a series of timely in-depth reviews written by researches in the field covering a range of the current topics in medicinal chemistry. Many clinically important drugs have been derived directly or indirectly from medicinal plants. Medicinal plants were used in India for centuries as an important therapeutic source for treating wide variety of ailments and have been found to be of immense global importance [14]. The genus *Polygonum* (Polygonaceae), comprising about 300 species, is distributed worldwide, mostly in north temperate climates, is interesting from both biological and phytochemical perspectives[15]. The medicinal plant *Polygonum glabrum* (Willd.) was selected based on its availability, therapeutic value for the screening of different active constituents present in the extract. In pharmacognosy, phytochemical analyses were carried out using modern analytical techniques such as fluorescence, UV- Vis, FTIR. Rahul showed maximum active phytoconstituents in the ethanolic and ethyl acetate extracts of *P. glabrum* [16]. Similar to Rahul, in the present study also, the chloroform, acetone and ethanolic extracts of *P. glabrum* illustrated maximum phytoconstituents. Sivakumar *et al* investigated UV and FTIR analysis only in chloroform extract of *P. glabrum* at 3136.6 cm^{-1} and confirmed chromophore group, hydrocarbon group, C-H bending group and carboxylic group. Contrary to Sivakumar *et al*. the UV and FTIR spectral analysis gave a maximum absorption in acetone and aqueous extract. The observed absorption band of present work between 516.92-3448.72 cm^{-1} indicates the presence of alkynes, alkenes, esters, ethers, aromatics, alkyl halides, aliphatic amines, carboxylic acids, aldehydes, nitro compounds, primary, secondary, tertiary amines and phenols.

The corresponding EDS spectrum of *P. glabrum* crude powder confirmed the occurrence of macro and micro elements like carbon, oxygen, chloride and potassium. In all these elements, carbon presented as high concentration while oxygen presented as moderate amount. Chlorine and potassium presented only a trace quantity. Potassium is necessary for muscle contraction (especially cardiac fiber), for the synthesis of some proteins and as an enzymic cofactor[17]. Chloride, in addition to potassium and sodium, assist in the conduction of electrical impulses when dissolved in bodily water.

CONCLUSION

The results of phytochemical and biological analysis showed that the metabolites present in the leaves of *P. glabrum* can be used to cure various diseases.These spectroscopic profiles will act as pharmacognostic marker to distinguish the medicinally important *P. glabrum* using relatively simple, cost-effective spectroscopic profile from its adulterants in the pharmaceutical industries.

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