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Pharmacognostic and Phytochemical Investigation of *Aitchisonia rosea*

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Aitchisonia rosea (Rubiaceae) is reported to have good medicinal values in traditional system of medicines. The current study was carried out to standardize the stem of this plant from pharmacognostical, phytochemical and physico-chemical point of view. The plant stem was found to be cylindrical in shape with yellowish brown colour. Microscopic evaluation showed the presence of thick cuticle, cortex, pericycle made up of sclerenchymatous cells, vascular bundles and clearly visible medullary rays. Cytomorphology of stem powder revealed hexagonal shaped parenchymatous cells, a group of fibers, reticulate type xylem vessels associated with calcium oxalate crystals and a covering trichome. The physico-chemical analysis of drug powder presented a composition of total ash 6.18%, water soluble ash 3.08%, acid insoluble ash 0.35% and loss on drying 7.15%. The Differential Scanning Calorimetry (DSC) thermogram of powdered drug showed the presence of incompatible crystalline and amorphous components with polymerization nature. Phytochemical screening of methanolic extract and its fractions revealed the presence of alkaloids, tannins, phenolics, flavinoids, saponins and terpenoids. Fourier Transform Infrared (FTIR) spectral evidence showed that stem powder probably contained methyl / aryl / ketonic / carboxylic acid / amine or some secondary amide group along with some –OH groups due to some alcohol or phenol. It was also observed that the plant was free of toxic heavy metals. The above parameters will be helpful for proper identification and establishment of standards for therapeutic use of *Aitchisonia rosea* stem.

Keywords: *Aitchisonia rosea*, Atomic absorption spectroscopy, DSC, FTIR, Pharmacognostic, Physicochemical, Phytochemical,

INTRODUCTION

Western medicines became the main system of healthcare but different communities still continue to benefit from the folk knowledge of the use of plants as medicines. Unfortunately most of this knowledge base has never been documented and thus threatened to disappear with the rapid demographic changes. It is the need of time to standardize these important medicinal plants and utilize them for treatment of different ailments.

Aitchisonia rosea Hemsl. ex Aitch belongs to plant family Rubiaceae. It is an important medicinal plant mostly distributed in hilly areas of Pakistan (Baluchistan) and Afghanistan. It is also found in Iran. The genus aitchisonia has only one specie i.e. *Aitchisonia rosea* (Ali & Nasir, 1989). *Aitchisonia rosea* has been collected by Aitchison in 1882 (Mozaffarian, 2006). In some areas of Baluchistan (Pakistan) this plant is used for the cure of some infectious skin diseases.

Two glycosides of iridoid nature, Aitchisonides A and B were isolated and identified from the n-

butanol fraction of *Aitchisonia rosea*. Deacetylasperulosidic acid and nepetanudoside B were also separated along with these two glycosides. These compounds were isolated from *Aitchisonia rosea* for the first time (Noor et al. 2009a).

Anthraquinone derivatives named Rosenones A and B were first time isolated and identified from ethyl acetate fraction of *Aitchisonia rosea* by using liquid column chromatography monitored by PTLC (Prepared Thin Layer Chromatography). Another compound1,3,6-trihydroxy-2-methylanthraquinone was also separated from this Aitchisoina specie (Noor et al. 2009b).

It is revealed from literature survey that pharmacognostic and pharmacological studies on *Aitchisonia rosea* have not been documented till to date. In this paper, we for the first time reported the pharmacognostic, physico-chemical, phytochemical parameters and biological potential of this plant species which might be utilized to standardize the plant for therapeutic purposes.

MATERIALS AND METHODS

Plant material

A. rosea was collected from hilly areas of Quetta, Balochistan, Pakistan. The specimen was further identified and authenticated by Taxonomist, Prof. Dr. Zaheer Ahmad Khan, Department of Botany, GC University, Lahore, Pakistan. Voucher specimen number 1911 was deposited in the Sultan Ayoub Herbarium, GC University Lahore for further reference. Stems and branches of plant were washed with distilled water to remove dust and other extraneous material. Fresh plant material was separated for organoleptic and morphological studies. Then they were spread on laboratory tables and dried under the shade for seven days at room temperature. The plant material was pulverized prior to use by using an electric mill.

Chemicals

All the chemicals and reagents used such as gallic acid (99%), folin-ciocalteu reagent and standard reference of K, Zn, Cu, Mg, Ca, Cr, Co, Pb metals were of analytical grade and purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Other chemicals and solvents like n-hexane, Chloroform, Ethyl acetate, n-butanol, Methanol, hydrochloric acid, nitric acid, Perchloric acid, iodine, Potassium iodide, ferric chloride, sodium hydroxide. Reagents like chloral hydrate, phloroglucinol, glycerin, safranin and light green were also of analytical grade.

Instruments

Instruments that were used for analysis during study include, Compound microscope (Labomed USA), Rotary evaporator (Heidolph, model LABORATA 4000, Schwabach, Germany), UV detection lamp 254 and 365 nm (Fisher Scientific Ltd, Pandan Crescent, UE Tech Park, Singapore), Digital camera (Canon 60d), UV/Visible spectrophotometer (Hitachi-UV-3200, Japan), FTIR spectrophotometer (IR Prestige-21, SHIMADZU Japan), Atomic absorption spectrophotometer (AA-6300 SHIMADZU Japan) and Thermal analyzer or Differential Scanning Calorimetry (SDT Q600 V8.2 Build 100, USA).

Solvent extraction and fractionation

The powdered plant material was extracted thrice with methanol by dipping for seven days. The methanol extract was concentrated to dryness under reduced pressure using rotary evaporator. The methanol extract was further fractioned using successive solvent extraction method with different polarity based solvents such as n-hexane, chloroform, ethyl acetate and n-butanol. After fractionation samples were concentrated to dryness under reduced pressure using rotary evaporator and stored in a refrigerator at 4°C, until used for analysis (Brain and Turner, 1975).

Pharmacognostic Evaluation

Macroscopic or organoleptic evaluation

Organoleptic characters like texture, colour, odour, taste, size, fracture and shape of fresh and dried stem of plant were observed.

Microscopic evaluation

Cytomorphology of stem was studied by preparing a transverse section of the stem and mounting the powdered drug.

A free handed transverse section of the stem was taken. In order to soften the hard stem, it was moistened by dipping in hot boiling water. Then the section was dehydrated with different grades of alcohol and stained with safranin and malachite green. The section was observed under microscope after being mounted with glycerin on a glass slide and photograph was taken by pohotomicrography (Youngken, 1950; Evans, 2003; Kokate et al. 2007).

For content uniformity, the drug powder was passed through sieve no. 40 and treated separately with different mounting media like

chloral hydrate, phloroglucinol-hydrochloric acid and chloral hydrate-glycerin solution to observe the microscopic characters of powder drug. These features were preserved as photographs (Youngken, 1950; Jackson and Snowdon, 1992; Harborne, 1998).

Physico-chemical analysis

The plant material was investigated for physico-chemical properties like total ash value, acid insoluble ash value, water soluble ash value, extractable matter and loss on drying by using prescribed methods (United States Pharmacopoeia, 2009; Anonymous, 1998).

Powdered drug was examined in day light, short and long wavelength ultra violet light for detection of florescent compounds after treatment with different chemicals (Kokoski et al. 1958; Chase and Pratt, 2008).

DSC was done to evaluate physiochemical nature of drug powder at a pre-defined rate of 10°C / min, over a temperature range of 40 – 600°C and inert environment was maintained by purging nitrogen gas at a rate of 20 mL / min as previously used by Pai et al. (2013). Graphs regarding heat flow and percentage weight of powder were obtained.

Phytochemical Evaluation

Qualitative phytochemical screening

Different chemical tests were conducted, using reported methods, to check out the presence of various phytoconstituents from methanol extract and its fractions (Edeoga et al. 2005; Evans, 2003; Kokate, 2007; United States Pharmacopoeia, 2009).

Quantitative phytochemical screening

The total phenolic contents, total alkaloid and glycoside contents present in the plant material were estimated by reported methods (Chaovanalikit and Wrolstad, 2004; Kokate, 2007).

FTIR spectroscopy

FTIR Spectrum of stem powder of plant was measured on spectrophotometer using thin film on Potassium bromide (KBr) disc and different functional groups were observed.

Heavy metal analysis

A heavy metal analysis was also done to check the quality and quantity of toxic metal in the plant by using Atomic absorption spectroscopy. For this purpose wet digestion method was used for sample preparation. 1 g dried powder of plant material was taken in a round bottom flask and 6 mL of conc. nitric acid was added to it. The mixture was kept for 16 hrs and then heated on hotplate at 80°C. Nitric acid addition causes the digestion of mixture. Further digestion was continued with 2 mL of perchloric acid (60%) until a clear solution was obtained by the same method as previously used by Rai et al. (2001). 10 mL distilled water was added drop wise and kept it for 4 hrs, then filtered into a volumetric flask and final volume was adjusted to 25 mL with the help of distilled water. Different metals were estimated in the sample by comparing with standard samples.

RESULTS

Percentage yields of plant extract and fractions

The Percentage yields (w/w) of crude methanol extract and its subsequent fractions are depicted in Fig. 1.

Pharmacognostic Evaluation

Macroscopic or organoleptic evaluation

Macroscopic or organoleptic characters of fresh and dried stem are tabulated in Table 1.

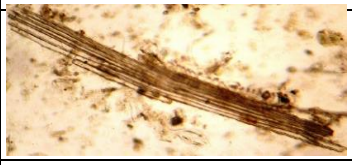
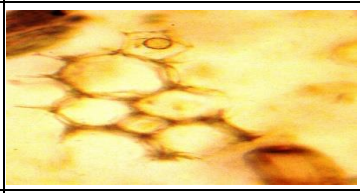
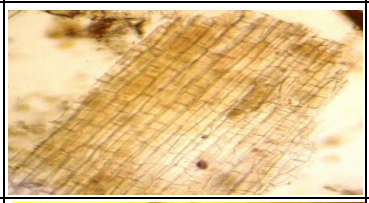
Microscopic evaluation

In transverse section of stem of plant, different histological features were observed under microscope as shown in Fig. 2. The stem powder microscopy is outlined in Table 2.

Table 1: Organoleptic evaluation of *A. rosea* stem

Parameters	Fresh stem	Dried stem
Shape	Cylindrical	Cylindrical
Colour	Yellowish brown	Yellowish brown
Odour	odourless	Irritant
Taste	Acrid	Acrid
Texture	Rough with spines	Rough with spines
External markings	Furrows and ridges	Furrows and ridges
Size (diameter)	Variable (1-2cm)	Variable (1-2cm)
Fracture	Hard and fibrous	Hard, incomplete
Internal colour	Yellow	Yellow

Table 2: Microscopic evaluations of *A. rosea* stem powder

Microscopic charchter		Identification		
Diagnostic part	**Figure**	**Chloral hydrate**	**Glycerine**	**Phloroglucinol HCL**
Parenvhyma cell		-	+	+
Group of fibers		+	+	+
Phloem tissue		+	+	+
Reticulate vessels		-	+	+
Covering trichomes		+	+	-

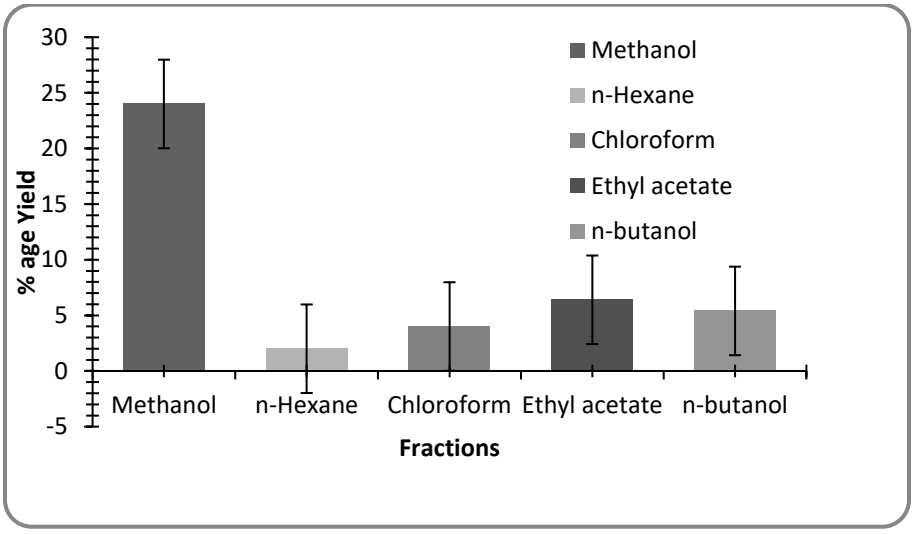


Figure 1: % Yield of Solvent Extracted Materials obtained from *A. rosea*

Physico-chemical analysis

The results of different physicochemical properties of powdered plant material are presented in Table 3. Behavior and fluorescence analysis of powder on treatment with different chemical reagents are compiled in Table 4.The physicochemical nature of B. calliobotrys powder was also evaluated by DSC (Fig. 2).

Table 3: Physicochemical properties of *A. rosea* stem powder

Parameters	% contents ± SD
Total ash	6.18 ± 0.16
Acid Insoluble ash	0.35 ± 0.05
Water soluble ash	3.08 ± 0.01
Loss on drying	7.15 ± 0.46
Alcohol soluble extractive	8.06 ± 0.35
Water soluble extractive	11.95 ± 0.76

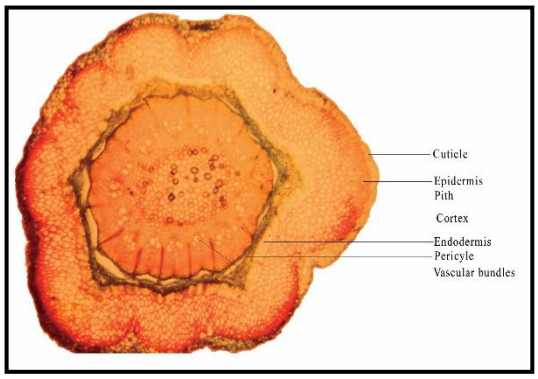


Figure 2: Transverse section of *A. rosea* stem

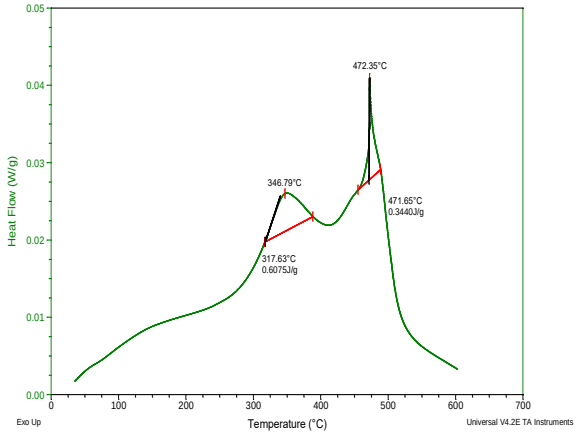


Figure 3: DSC Thermogram of powder of *A. rosea*

Phytochemical Evaluation

Qualitative phytochemical screening

The qualitative phytochemical analysis was used to evaluate the occurrence of different chemical constituents like alkaloids, glycosides, tannins, saponins, terpenoids, fats, phenols and flavonoids in methanol extract and its various fractions. The findings are shown in Table 5.

Quantitative phytochemical screening

The values of total phenolic contents (TPC) were found to be 16.20 mg/g as gallic acid equivalent of dry plant material. The percentages of Alkaloids and glycosides contents were 2.60 and 0.32 respectively.

FTIR spectroscopy

FTIR Spectrum of stem powder of plant revealed different functional groups (Fig. 4).

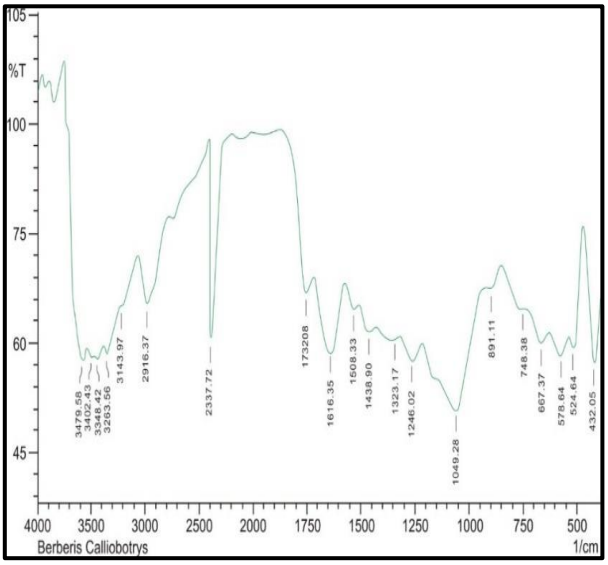


Figure 4: FTIR Spectrum of stem powder of *A. rosea*

Heavy metal analysis

The percentages (ppm) of heavy metals in A. rosea by using atomic absorption spectroscopy are shown in Fig. 6.

Table 4: Fluorescence analysis of *A. rosea* powder on treatment with different chemical reagents

Treatment	Visible light	U V Light	
		Short Wavelength (254 nm)	Long Wavelength (365 nm)
Powder (P) as such	Yellowish brown	Greenish yellow	Blackish brown
P + water	Light brown	Greenish Yellow	Blackish brown
P + Aniline	Reddish Brown	Red	Chocolate brown
P + Chloroform	Light brown	Greenish Yellow	Dark yellow
P + Methanol	Dark brown	Greenish yellow	Blackish brown
P + 5 % Ferric chloride	Light green	Dark green	Dark green
P + Iodine Solution	Dark Red	Brown	Black
P + 50 % KOH solution	Orangish brown	Blackish green	Black
P+ 5 % NaOH solution	Light yellow	Yellowish green	Brownish green
P + 50 % Sulphuric acid	Light brown	Greenish black	Dark brown
P + 66 % Sulphuric acid	Light brown	Blackish brown	Black
P + Dilute HCl	Yellowish orange	Greenish black	Blackish brown
P+ 50 % Nitric acid	Chocolate brown	Dark yellow	Black

Table 5: Qualitative phytochemical analysis of *A. rosea* various fractions

Identification tests	Methanol	*n*-hexane	Chloroform	Ethyl acetate	*n*- butanol
Carbohydrates					
Molisch test	**+**	-	-	+	+
Benedict's test	+	**-**	**-**	**-**	**+**
Iodine test	**-**	**-**	**-**	**-**	**-**
Proteins					
Ninhydrin test	-	-	-	-	-
Biuret test	-	-	-	-	-
Millons test	+	-	-	+	+
Alkaloids					
Mayer's test	+	-	**+**	+	+
Dragendorff's test	**+**	-	+	+	+
Wagner's test	**+**	-	-	+	+
Hager's test	**+**	-	**-**	+	+
Glycosides					
Keller-Killiani test	**+**	**+**	+	+	+
Bromine water test	**+**	**+**	-	+	+
Tannins, Phenols					
Ferric chloride test	+	-	-	+	+
Fats and Fixed Oils					
Saponification test	**+**	**+**	**-**	**+**	**-**
Flavonoids					
Lead acetate test	**+**	**-**	**-**	**+**	**+**
Alkaline reagent test	**+**	**-**	**-**	**-**	**-**
Saponins					
Foam test	**+**	**+**	**-**	**+**	**-**
Terpenoids					
Salkowski test	**+**	**+**	**+**	**-**	**-**
Liebermann Burchard test	**+**	**+**	**-**	**-**	**+**

DISCUSSION

Herbal drugs have gained massive importance during last few decades and there has been an ever increasing demand for these drugs due to their various therapeutic benefits. So maintenance of proper quality control profile is necessary as adulteration may result from improper and insufficient knowledge with respect to different geographic regions and conditions in these regions. In the current study, pharmacognostic, physico-chemical and phytochemical parameters were evaluated which might be utilized to standardize the plant.

The maximum percentage yield was observed for methanol crude extract while the minimum for n-hexane fraction. It could thus be concluded that the powdered drug of A. rosea contained greater proportion of polar compounds as compared to non-polar components. The methanol is a good solvent to extract various phytoconstituents from plant sources (Rizwan et al. 2012; Riaz et al. 2012b).

Organoleptically, the yellowish brown colour stem was almost cylindrical in shape. The surface of stem was rough with furrows and contained spines. It was 1-2 cm in diameter. The fractured surface was yellow in colour.

The transverse section of stem was almost circular in outline. The epidermis was visible as a thick cuticle and comprising few layers of cork cells. Ground tissue was present below the epidermis and was found to have been made up of several layers of collenchymatous cells. Sclerenchymatous cells form the cortex comprising sclerenchymatous fibres. Vascular bundles were present inside the pericycle. Inner to vascular tissues, pith was present which occupied the central region. It was composed of cells of parenchymatous cells, with intercellular spaces. Extensions of these cells into the areas in between vascular bundles formed medullary rays which were clearly observed. Simple starch grains and calcium oxalate crystals were present in parenchymatous pith.

The stem powder was yellowish brown in colour and has a pleasant odour. Its taste was slightly bitter. Three mounting solutions i.e., chloral hydrate, chloral hydrate-glycerine and phloroglucinol-HCl solutions were used for this purpose. The main reason for utilizing the chloral hydrate as mounting media is that majority of powders could be best observed in this mount. The main disadvantage of this mount is the crystallization of chloral hydrate itself, particularly in winter season. To overcome this problem, Chloral hydrate-Glycerine mount was used. This inhibited the formation of crystals of chloral hydrate. Phloroglucinol mount in the presence of hydrochloric acid, was used to identify the lignified tissues. Cytomorphology of powder revealed hexagonal shaped parenchymatous cells. A group of fibers along with starch grains were present in chloral hydrate-glycerin and phloroglucinol-HCl mounted powder. The reticulate type xylem vessels associated with calcium oxalate crystals were also observed. Phloem tissues were viewed in longitudinal manner and a covering trichome was seen in the powdered drug. The above mentioned tissues were identified by comparing with the tissues outlined in other standard work (Jackson and Snowdon, 1992; Youngken, 1950; Harborne, 1998).

The physico-chemical contents like total ash, water soluble ash, acid insoluble ash, loss on drying and extractive values obtained were found within specified official limits which reflects that the care was taken during collection, drying and storage of plant material. Total ash value is required for the information about inorganic compounds such as carbonates, phosphates, silica and silicates that are naturally found in drug or added separately as adulterant. Acid insoluble ash gives the percentage of dirt and sand. The moisture content plays significant role in efficacy and stability of powdered herbal drugs. This should be controlled to prevent chemical degradation and microbial invasion. Water was found to be greater extractive potential than alcohol. This data would be helpful for determination of purity and identification of genuine sample of stem of *A. rosea*. Fluorescence is an important phenomenon exhibited by various phytochemicals under UV light. The powder showed different behavior and fuorescence on treatment with different chemical reagents. This is also a useful parameter for setting standards for herbal drugs.

The DSC thermogram of *A. rosea* powder showed temperature range from 254.56°C to 472.35°C. A smaller step height at temperature 254.56°C revealed the absence of plasticizers in the powder sample. Generally Incompatible mixture shows two transition states in the heat flow graphs and compatible mixture shows single transition states. The thermogram of powder showed more than one transition states indicated that the components of the powder were incompatible and could be separated easily as mentioned by Kennedy et al. (2010). Cross linking behavior of the components within the mixture at

temperature 472.35°C indicated the polymerization nature of the components within the drug powder as previously discussed by Lee *et al.* (2008). The endothermic (346.79°C) and melting (317.63°C) peaks in the graph represented the presence of both crystalline and amorphous nature of compounds in the drug powder (Dai et al. 2008).

The analyzed phytoconstituents were found to be present but some were absent in certain plant fractions. It might be due to the effect of type of solvent used. The phytochemicals are produced in many plant species and exhibit pharmacological and biological activities. Antioxidant and antimicrobial activities were reported in various plants due to the presence of tannins, alkaloids, steroids, saponins and terpenoids (Riaz et al. 2012a; Erol et al. 2010). Terpenoids are found in various plant parts having many therapeutic uses like antimicrobials and also used in many pharmaceutical preparations (Michael, 2009). The values of total phenolic contents (TPC), alkaloids and glycosides contents confirmed the presence of these constituents in the plant material.

FTIR analysis of powdered drug is a useful parameter for evaluation of nature of phytochemicals present in the drug material. In FTIR spectrum a band at 3400-3200 cm^{-1} was due to -OH absorption which might be due to some alcoholic or phenolic group, or some complex polyhydroxy groups of tannins. The broadening of this band emphasized the stretching vibration of -OH with intra-molecular H-bonded at OH. The bands at 2916 cm^{-1} and 2338 cm^{-1} showed the C-H stretching vibration present in alkane, alkene or alkyne. Often such strong bands are shown by the methyl, methylene or aryl groups resulted from symmetrical and asymmetrical stretching C-H modes. A strong peak at 1616 cm^{-1} indicated the presence of a carbonyl group (C=O) which might be a part of aldehyde / ketone / carboxylic acid or some acidic anhydride. Strong peak at 1616 cm-1 also revealed the presence of conjugation with double bond might be due to an aromatic ring. The presence of carbonyl group was further confirmed by strong peaks at 1049 cm^{-1}. The band at 2916 cm^{-1} also indicated the presence of N-H symmetrical and asymmetrical stretching vibration due to –NH2, =NH, ≡N or –CONH group in the molecule (William and Flaming, 1980; Silverstein, 1981). The available spectral evidence showed that stem powder of B. calliobotrys probably contained methyl / aryl / ketonic / carboxylic acid / acid anhydride / amine / nitrile or some secondary amide group along with some –OH groups due to some alcohol or phenol.

The concentrations of all heavy toxic metals in the plant were within official limit and as per WHO guidelines (Markert, 1994; Anonymous, 1998). It could be concluded that the plant is not toxic and could be safely utilized for therapeutic purposes.

CONCLUSION

The study of phytochemical and pharmacognostical features of *A. rosea* had shown the standards, which will be effective parameters in the identification and recognition of its purity and genuineness. Physicochemical parameters such as pH, acid values, extractive values and fluorescence analysis are indicators of the quality of material. These pharmacognostical evaluations and physicochemical characterization all are anatomical features helpful for a researcher in their research work for authenticity of this plant material.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

SR designed the whole research plan, SI fulfilled the all required procedures for research. SA wrote the whole manuscript. NA help in table formulation, SR and SR helped in calculation of Mean and SD and help in cross checking.

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