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Original Article

Pharmacognostic Features, Preliminary Phytochemical Screening and *in Vitro* Antioxidant Studies of Indigenous Plant of Lahore

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ABSTRACT

Introduction: *Periploca aphylla* is belongs to family *Asclepiadaceae* and it is an important traditional plant which has been utilized for decades for its local uses.
Methods: This research work included pharmacognostic studies, microscopic evaluation, phytochemical and physiochemical tests for the effective identification and characterization of plant. Transverse section of plant shows the arrangement of cells. Fluorescence analysis shows colors in light of different wavelength. Fluorescence analysis was performed with various reagents and chemicals to observe characteristic pattern and colors for the identification and proper authencity of plant and it was reported in this paper.
Results: Phytochemical studies show the presence of alkaloids, saponins, flavonoids and tannins. Powder study shows lignified fibers, spiral vessels and lignified tracheid. In this study Fourier-transform infrared spectroscopy was used for the identification of plant and for the effective recognition of biological composition of plant. The IR Spectra were performed and reported in this paper. All these parameters are necessary for the characterization and identification of plant and a new doorstep to find out any adulteration in the plant.
Conclusion: Anti-oxidant *in-vitro* studies were performed for the evaluation of any anti-oxidant property which provided a brief description about its pharmacological effects regarding rancidity and also a description about its further usage in any of anti-oxidant formulation.
Keywords: *Asclepiadaceae*; Phytochemical studies; *Periploca aphylla*; pharmacognostic studies.

1. Introduction

It is an important fact of life that plant has an immense effect on the health of humans and also curative tendency for researchers. Historically, Pakistan has a very rich flora and this historical background supports the standardization of few locally occurring plant in this present research work. In conventional medicines, plants are used for their effective raw materials [1-3]. In this case *Periploca aphylla* is an important plant which is abbreviated as *P.aphylla* and has been used since ancient times for their local recipes.

This study included various physiochemical as well as phytochemical evaluation which discloses the effectiveness of local use and also presented a milestone for the medicinal properties of the plant. Fresh plant *P.aphylla* was collected in this study and was standardized with the help of pharmacognostic studies such as microscopic and organoleptic evaluation, histological studies, fluorescence analysis, proximate analysis and phytochemical analysis. Microscopic evaluation of *P.aphylla* powder was subjected for the diagnostic characteristics of individual stem and leaves [4]. It belongs to family Asclepiadaceae and the plant is commonly found in the form of shrub sometime in the form of herb. This genus is widely distributed in Africa and Asia; seven of the species are present in Asia. Series of *Periploca* is Afro- Eurasian specie group. The region of Monocoronata has the least species in this genus [5]. All of the members are xerophytes and have the highest degree of modifications. The latex of this plant is a good remedy for skin ailments especially for the healing of wounds. Also, decoction of the plant has been used for the cure of fever and headache [6]. One of the local uses of P.aphylla is that it is used as diaphoretic stimulant, emmogogue and tumor. Some reported effects of the plant are antidiarrheal and laxative activity, antifungal activity and analgesic and neuropharmacological effects [7]. Despite its vast uses and pharmacological actions, no comprehensive information is available about pharmacognostic features, morphology and physiochemical properties. Therefore, this study offered comprehensive information about its pharmacognostic features used for the authencity and identification of plants which is the necessary tool in any research and development project. This is the baseline which is set for any development that material should be pure and adulterant free. This study provided the major identification pattern for the new developers [8].

2. Material and Methods

2.1. Plant materials

The plant was collected from Botanical garden of Government College University, Lahore and was authenticated by Sir Zaheer Department of Botany, Government College University, and Lahore Pakistan, based on authenticity established by Gamble, Benthum and Hooker. A specimen of plant was deposited in herbarium of Government College University, Lahore under Voucher Specimen No: GC. Herb. Bot. Aerial parts were separated and all parts were dried under shade and then preserved in amber colored glass bottles.

2.2. Chemicals, reagents and instruments

Analytical grade Acetonitrile, Methanol, n-Hexane, ethanol, acetic acid, lead acetate, ammonia, hydrochloric acid, sulphuric acid, safranin, bromine water, chloroform, fast green, ferric chloride, iodine, nitric acid, distilled water, sodium hydroxide solution, Wagner's reagent, Dragendroff's reagent, Million's reagent, Molisch's reagent, Quercetin, Ascorbic Acid and ethyl acetate were used.

Instruments which were used included UV-Vis spectrophotometer (Shimadzu), Muffle Furnace, Ultraviolet Lamp, Electron Microscope, and Fourier-transform infrared spectroscopy (FTIR (Shimadzu) were used.

2.3. Organoleptic evaluations

Different organoleptic evaluations of fresh and dry state of whole plant were observed and were determined according to the protocols [9].

2.4. Powder study

Chloral hydrate solution was used for the observation of powder drug using microscope. Slides of powdered leaf and stem were prepared according to the prescribed procedures. Photographs were taken, using microscope by means of digital camera [10, 11].

2.5. Transverse Section Cutting:

Fresh leaf and stem were preserved in formalin: acetic acid: 70% alcohol (5:5:90) for 24 hours transverse sections were made by commonly used blade. Sections were stained with safranin and fast green and observed under microscope. The photographs were taken [12, 13].

2.6. Fluorescence analysis

Fluorescence analysis was carried out by using different reagents and ultraviolet light of different wavelength as well as with daylight. Different colors were noted [14].

2.7. Physicochemical analysis

Powdered samples of *P.aphylla* were subjected to physicochemical analysis AS their extractive values and with total ash, water soluble ash, and acid insoluble ash study.

3. Results and Discussion

3.1. Organoleptic evaluation

Different organoleptic evaluations of fresh and dry state of whole plant were observed and determined (Table 1).

Table 1. Organoleptic evaluations of fresh and dry state of whole plant

Features	*P.aphylla*	*Observation*
Stage	Fresh	Dry
Taste	Bitter	Bitter
Texture	Smooth	Smooth
shape	Soft branches with no leaves	Soft branches with no leaves
odor	Odorless	Odorless
color	Green	Light green

3.2. Microscopic evaluation of P.*aphylla* plant

Powder microscopy of the plant shows Starch granules, Phytolith, Group of fibers with associated vessel, Parenchyma, Micro rosette crystal of calcium oxalate, Spiral vessel, Single trichome, Branched covering trichome, Wrinkled walled parenchyma, Fibers with associated parenchyma, thin walled sclereid, Bundle of fibers (Figure 1,2, & 3).

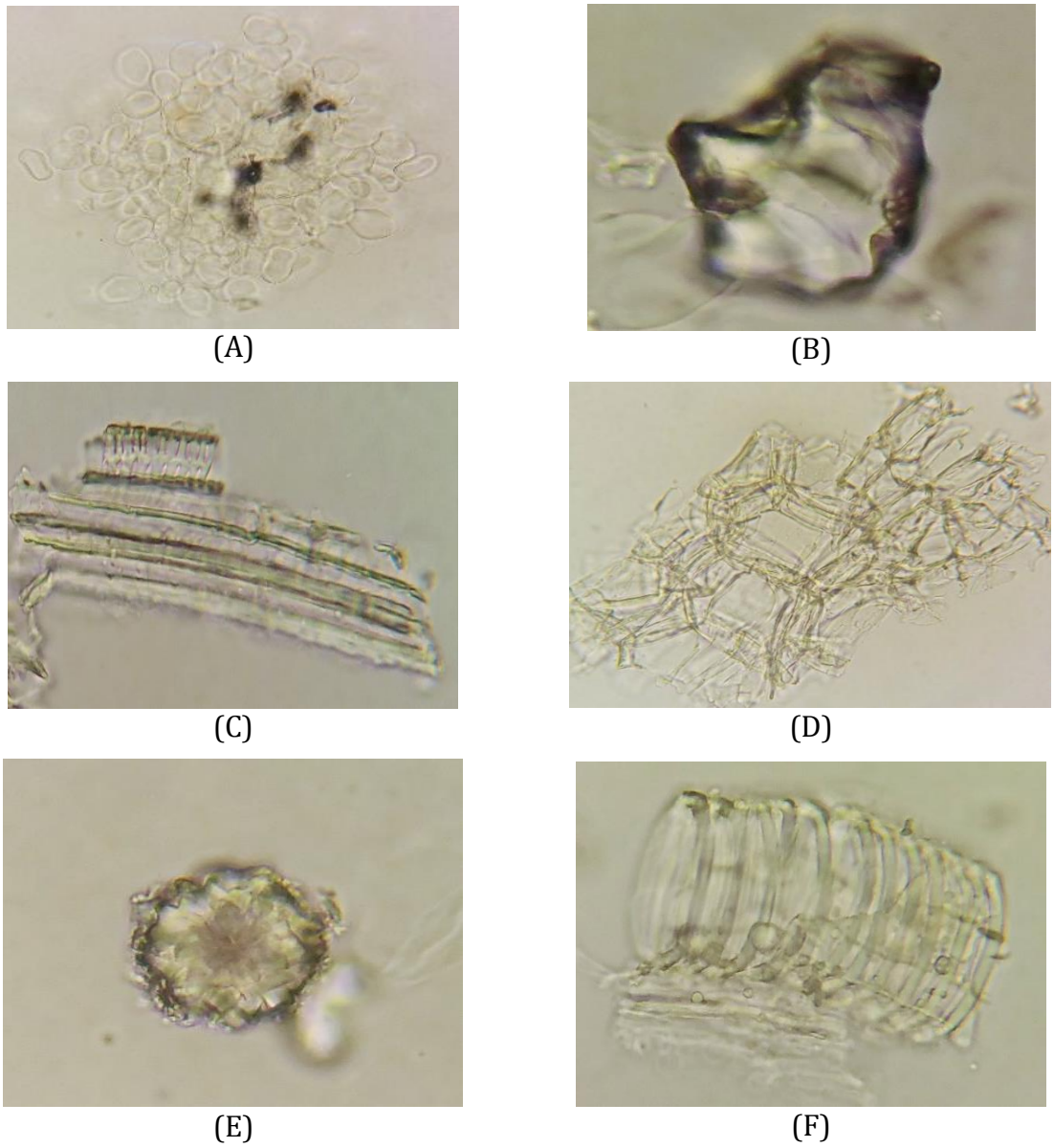

Figure 1. Microscopic evaluation of P.aphylla plant, (A) Starch granules,(B) Phytolith(C) Group of fibers with associated vessel (D) Parenchyma (E) Micro rosette crystal of calcium oxalate(F) Spiral vessel

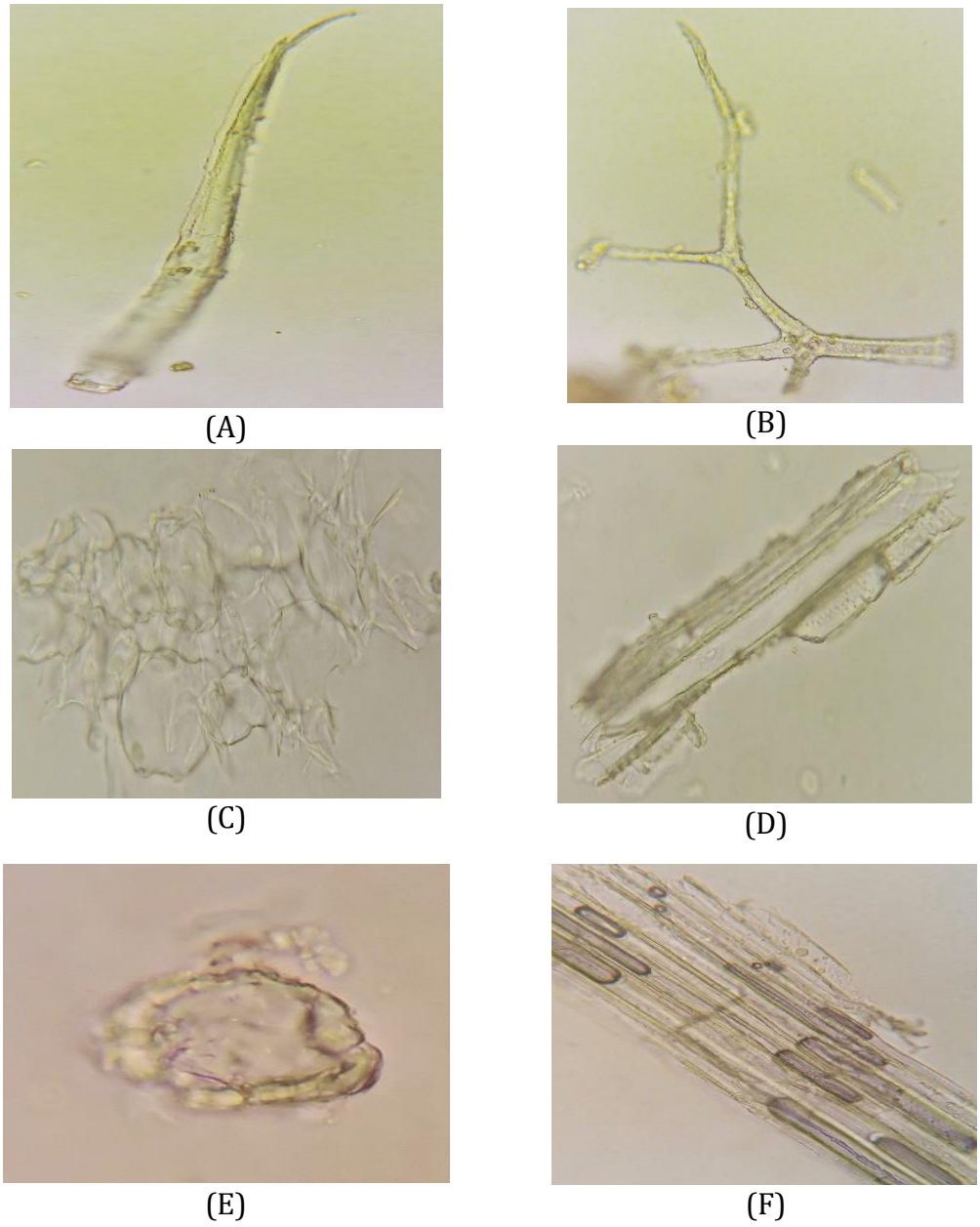

Figure 2. Microscopic evaluation of P.aphylla plant (2) (A) Single trichome (B) Branched covering trichome(C) Wrinkled walled parenchyma (D) Fibers with associated parenchyma (E) Thin walled sclereid (F) Bundle of fibers

3.3. Transverse section cutting of whole plant

Section cutting of the plant has been done and viewed under electron microscope and the pictures were taken and marked as below:

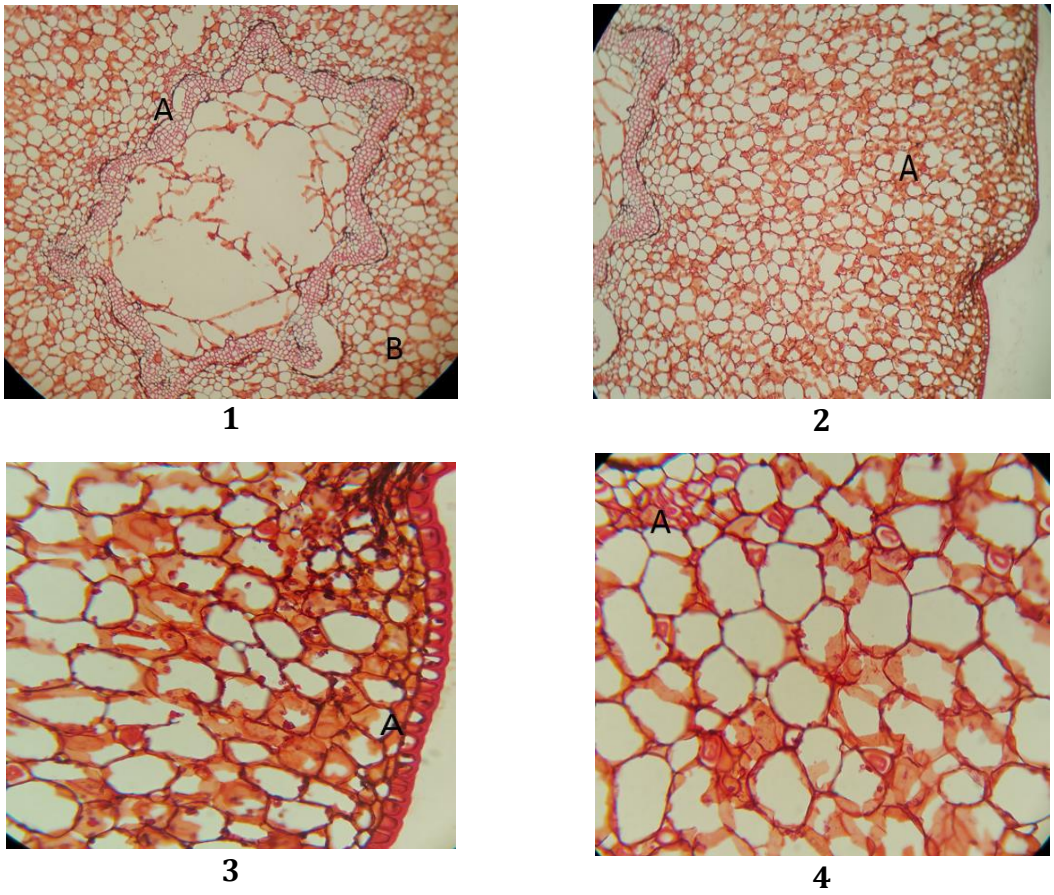

Figure 3. A) Parenchyma cells, B) collenchyma cells, 2) A) collenchyma cells, 3) A) collenchyma cells, 4) A) vascular bundles

3.4. Fluorescence analysis of powder p.*aphylla* plant

Fluorescence analysis of whole plant was done with different reagents under visible, short wavelength and long wavelength and the following colors were observed (Table 2).

Table 2. Fluorescence analysis of *P.aphylla* whole plant

Sr. no	Reagent's used	Visible range	Short wavelength	Long wavelength
1	Powder +$FeCl_3$	Green	Orange brown	Black
2	Powder+HCl	Orange green	Brown	Green
3	Powder+Conc HNO_3	Yellow	Dark orange	Light green
4	Powder + NaOH	Brown	Orange brown	Green
6	Powder+Conc H_2SO_4	Black	Brick Red	Pale green
7	Powder+Bromine water	Brown	Light brown	Sea green
8	Powder+Chloroform	Brown	Orange	Grey
9	Powder+Methanol	Green	Yellow Orange	Tea pink
10	Powder+Acetic Acid	Yellow brown	Brown	Off white
11	Powder+Iodine	Brown	Orange brown	Green Black
12	Powder+NH_3	Brown	Pink	Light green
13	Powder+water	Brown	Pink	Light green

3.5. Physiochemical analysis or proximate analysis

Physiochemical analysis or proximate analysis including total ash, water soluble ash, acid insoluble ash, swelling index, moisture contents, foaming index of P.*aphylla* whole plant were determined and their values are in presented in Table 3, respectively.

Table 3. Proximate analysis of the plant

Sr.No	Physico-chemical Parameters	*P.aphylla*
1	Total Ash(less than 13%)	4.60%
2	Water soluble Ash(less than 10%)	1.01%
3	Acid insoluble Ash (0.5-5.5%)	5.11%
4	Swelling Index(up to 5 mL)	2.1 mL%
5	Moisture Contents (8-14%)	1.80%
6	Foaming Index (100-1000)	>100

3.6. Phytochemical analysis of *P.aphylla:*

Phytochemical tests are performed on the methanolic extract of the plant and the determination of Alkaloids, Carbohydrates, and Phenols. Flavonoids**,** Tannins, Lipids and saponins with various tests were performed on *P.aphylla* plant and the results are described in Table 4.

Table 4. Phytochemical constituents of *P.aphylla*

Sr.No	Phytochemical constituents	Tests Performed	Results
1.	**Alkaloids**	Mayer's Reagent	Present
		Wagner's Reagent	Present
		Hager's Reagent	Present
2.	**Carbohydrates**	Benedict's Tests	Absent
		Fehling's Test	Absent
3.	**Phenols**	Ferric Chloride test	Absent
4.	**Flavonoids**	Lead Acetate Test	Present
5.	**Tannins**	Gelatin Test	Present
6.	**Lipids**	Spot Test	Absent
7	**Saponins**	Foam Test	Absent

3.7. Total phenolic content

Total phenolic content of the extracts of plant was measured by folin-ciocalteau method and results were calculated by applying equation obtained by standard Gallic acid curve. The obtained results were in the following graphs (Figures 4 & 5).

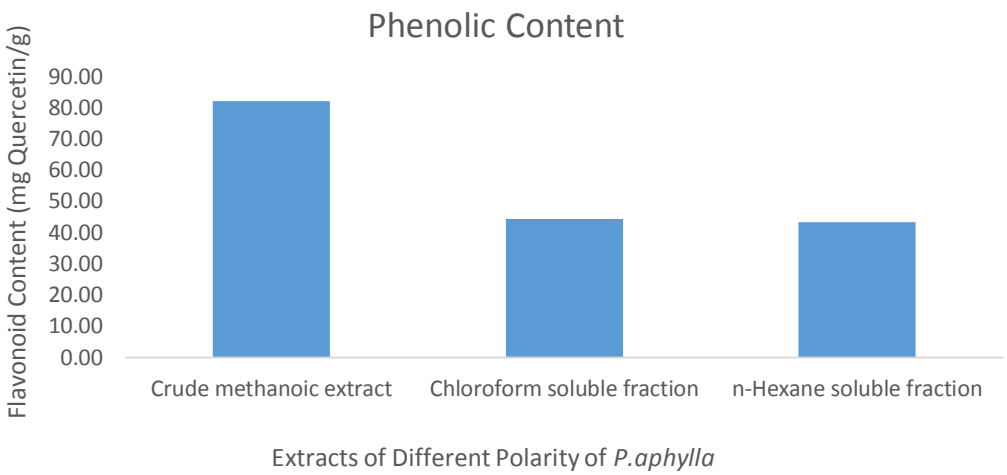

Figure 4. Extracts of P.*aphylla*

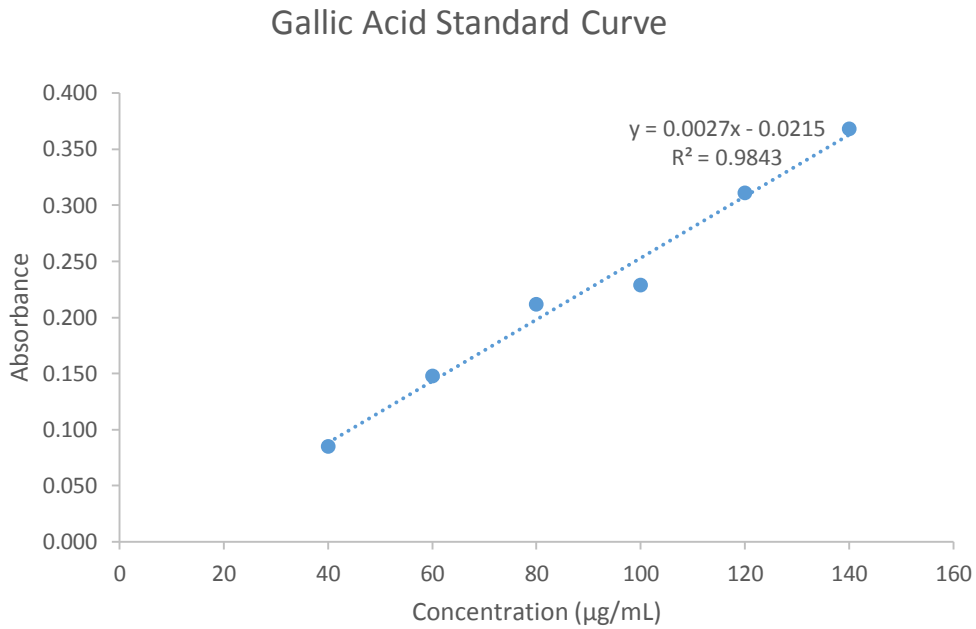

Figure 5. Gallic acid standard curve

3.8. Total flavonoid content

Total flavonoids contents of the extracts of plants were evaluated by applying the standard quercitin curve equation in the terms of the Quercitin equivalent (Figures 6 & 7).

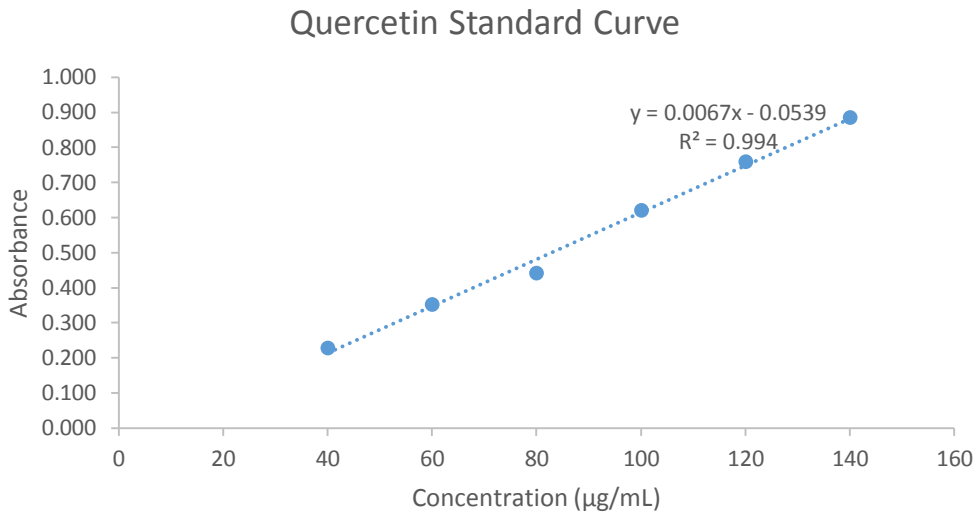

Figure 6. Quercetin Standard Curve

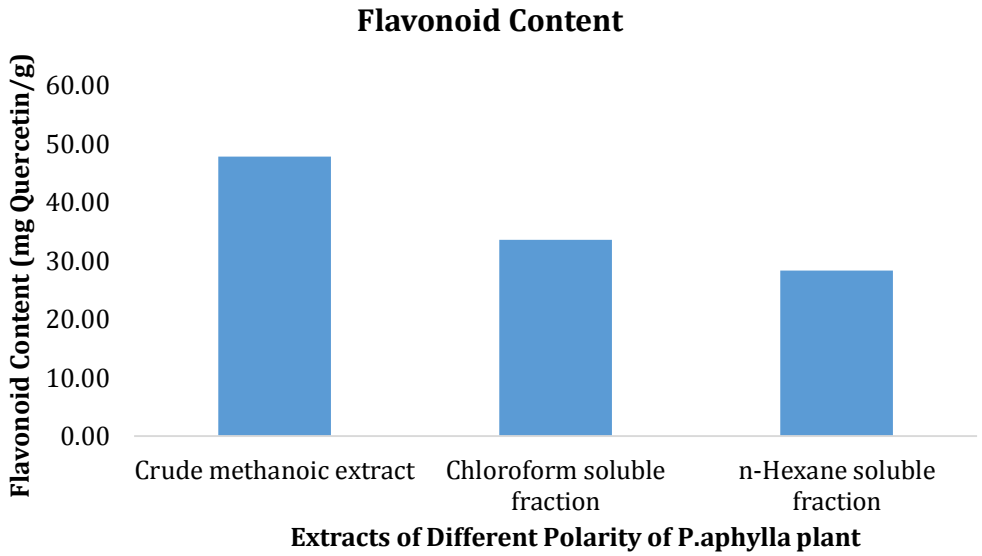

Figure 7. Extracts of P.*aphylla* plan

3.9. Fourier transform infrared spectrophotometer (FTIR)

Technique of FTIR has been used for the determination of functional groups and for the specific bonds of the plant powder. This is also useful for the molecular characterization of any plant part different peak values of pant material show functional groups presence as well as specific bonds. It also shows the shape of the peak which is helpful for the identification of bonds and groups present in biological fragment (Figure 8).

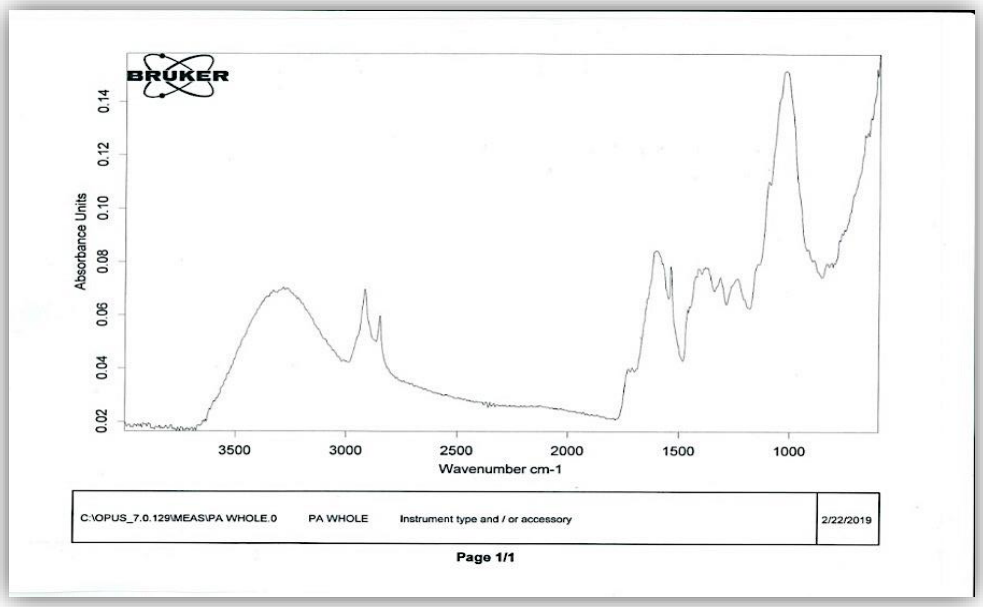

Figure 8. FTIR scan of P.aphylla plant

The plant shows broad peak at 3600 and it is broad, and the other peaks are at 1600 and at 1100 with a narrow and sharp spectrum.

3.10. Antioxidant activity

In vitro antioxidant activity by DPPH free radical scavenging method:

Antioxidant activity of plant extracts in different chemicals (methanol, n-hexane and chloroform) was determined according to method which is described in the reference is used with slight modifications (Table 4).

Table 4. Antioxidant activity of plant extracts

Concentration (µg/mL)	200	400	600	800
Ascorbic acid	74.97	77.46	83.71	86.24
Methanol	53.50	55.94	58.69	62.40
n- hexane	51.51	52.07	57.55	59.36
Chloroform	53.08	54.30	62.27	63.58

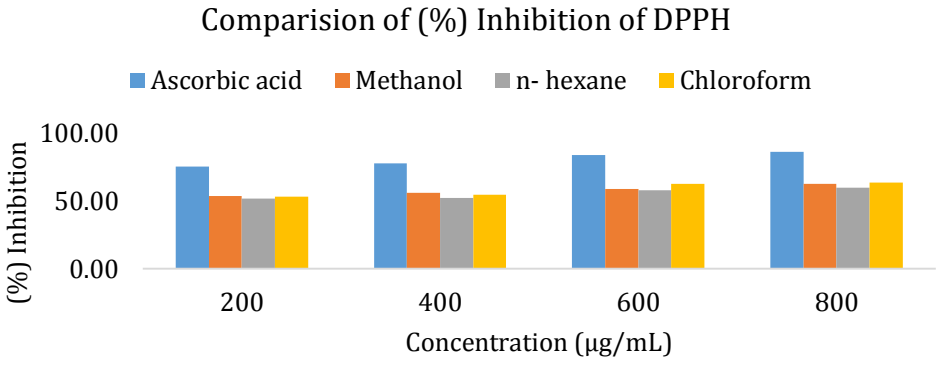

Figure 9. Comparison of (%) Inhibition of DPPH

4. Conclusion

The pharmacognostic study of *Periploca aphylla* represents the standards which are useful for its identification and authencity. The phytochemical profile of *Periploca aphylla* determined were found to be positive for most of the secondary metabolic groups. Characteristic colors and fluorescent properties were recorded and they could be used for proper identification and authentication this could also be used to check adulteration in any drug powder. Powder study and transverse sections of plants showed the exact role of part of plant and also played a helpful role for the biological researchers [11]. In any mixture of drugs, studying fluorescence helps to identify specific drug or plant. Fourier-transform infrared spectroscopy is an accurate technique for the identification of molecular structure of biological fragment and it shows the graphical representation of plant which is helpful in the authencity identification of plants. The IR-Spectra also shows the show the wavelength and shape of peak for the identification of functional groups which are present in the plant. Furthermore, phenolic content and flavonoid content were determined which were found to play a key role in the validation of *Periploca aphylla* and in in-vitro anti-oxidant studies which would play an important role in the development of any pharmacological activity for new researchers and in new formulations.

Abbreviation

Not applicable

Conflict of Interest

Authors declare there is no conflict of interest in this research study

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All data generated during this study are included in this published article.

Ethics approval and consent to participate

No human or animals were used in the present research.

Ethics declarations.

The authors declare no conflict of interest in financial or any other sphere. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Source of Funding

No funding was received against this research study.

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