

Original Research Article

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Comparison of Quality Control Parameters of Leaf and Stem of *Phyla nodiflora* L. Greene (Verbenaceae)

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ABSTRACT

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Phyla nodiflora L. Greene (Verbenaceae) is a medicinal plant commonly known as Ratoliya in Gujarati and Bhujokra in Hindi. Traditionally it is used to cure many diseases like diarrhoea, ulcers, asthma, bronchitis, knee joint pain, gonorrhoea and also there are many reported pharmacological activities like antibacterial, antifungal, antiinflammation, antiulcer, antidiuretic, etc. Thus, it is very essential to lay down quality control parameters so that the therapeutic efficacy of the plant can be maintained. In order to achieve this, in the present work, *Phyla nodiflora* leaf and stem were evaluated for their physicochemical, phytochemical and pharmacognostic parameters. For phytochemical analysis, different phytoconstituents like saponins, tannins, glycosides, phenols, triterpenes, flavonoids, steroids, etc. were evaluated. For physicochemical analysis, different ash values, extractive values and loss on drying was determined following all standard procedures. Both leaf and stem were rich in phenols, flavonoids and alkaloids; however, leaf had steroids and stem had saponins in maximum amount. The ash values were more in leaf than in stem while loss on drying was almost same (> 10%) in both the parts. Likewise, extractive value was maximum in methanol and minimum in petroleum ether. The diagnostic characters laid down in this study will be helpful for its correct identification and ensure its purity. They are the most simple, easy and cheapest quality control parameters.

Introduction

Herbal drugs referred as plant based materials, involves the use of whole plant or parts of plant to treat injuries or illness (Edeoga *et al.*, 2005). Medicinal plants occupy a significant position in community because they play a vital role in human health. The medicinal value of the plants lies in some chemical substances that cause a definite action on

human body. Medicinal plants are widely used by the traditional medical practitioners for counteracting various diseases in their routine practice. Natural products from plants have been the basis of the treatment of human disease. Today 80 % of population of developing countries still rely on traditional medicine based on plant species for their primary health. Herbal medicines are currently in demand and their popularity is increasing

day by day (Verma and Singh, 2008). Herbal drugs are commonly used throughout the world because of lower side effects, easy availability, low cost and its effectiveness (Nasri and Shirzad, 2013).

Since many years, humans have relied on nature for their basic needs for the production of food-stuffs, shelters, clothing means of transportation, fertilizers, flavours, fragrances, and medicines. Plants have formed traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies (Gurib-Fakim, 2006). The search for new pharmacologically active agents obtained by screening of plant extracts has led to the discovery of many clinically useful drugs that play a major role in the treatment of human diseases (Shu, 1998). Plants have many therapeutic activities like antibacterial (Kumar *et al.*, 2014; Rakholiya *et al.*, 2014a; Donga *et al.*, 2017a), antioxidant (Rakholiya *et al.*, 2014b; Donga *et al.*, 2017b), anti-inflammatory (Zhen *et al.*, 2016), diuretic (Chen *et al.*, 2014), hepatoprotective (Muthaiyan *et al.*, 2015), etc. Despite taxonomical classification, herbal drugs can be classified according to their morphological character. For e.g. seed drug shows antioxidant activity (Hussain *et al.*, 2016), leaf drug shows – antidiabetic activity (Arumugam *et al.*, 2008), bark drug shows- antibacterial activity (Muthkar *et al.*, 2006) flower drug shows - anti-inflammatory activity (Balkan *et al.*, 2018).

Phyla nodiflora L. Greene is an important member of the family Verbenaceae. This family includes 34 genera. Plants from Verbenaceae family are well known for their use in traditional medicinal system of various countries. *Phyla* is used as the traditional medicine for the treatment of various skin diseases and is used as folk cosmetics among the tribal communities of Pakistan (Abbasi *et al.*, 2010). Some of the reported

pharmacological activities of *Phyla nodiflora* are given in Figure 1. Medicinal plants are in use since ages however they are not becoming as popular as they deserve mainly because there are no stringent quality control methods for herbal drugs. The importance of herbal medicine is questioned and quite often they are prone to adulteration and substitution intentionally or unintentionally. Thus, quality control and standardization is essential at each step for e.g. Raw material standardization, manufacture process standardization and standardization of finished product. This will ensure the quality, safety and efficacy of herbal drugs.

Considering the above, in the present work, an attempt has been done to lay down quality control parameters of *Phyla nodiflora*. It involves pharmacognostic standardization. The pharmacognostic standardization parameters which are generally done are organoleptic characters, macroscopic study, microscopic study, physicochemical analysis, phytochemical analysis, fluorescence analysis (Chanda, 2014). The parameters evaluated are given in Figure 2.

Materials and Methods

Plant collection

Plant *Phyla nodiflora* (L.) Greene was collected in August, 2017 from Porbandar, Gujarat, India. The plant parts leaf and stem were separated, washed thoroughly under tap water, Shade dried and homogenized to fine powder and stored in closed container for further studies.

Pharmacognostic study

Macroscopic studies

Pharmacognostic study was done by organoleptic evaluation. The morphological

features of different parts of the plant were observed under magnifying lens. Macroscopic characters were studied using standard methods (Khandelwal, 2008). Photographs at different magnifications were taken by using digital camera.

Microscopic studies

Microscopic studies were carried out by preparing thin sections of different part of different plants. The thin sections were further washed with water, stained with safranin, fast green and mounted in glycerine for observation and confirm its lignifications (10x, 40x). The powder microscopic studies were also carried out and the specific diagnostic characteristic features were recorded (Tyler *et al.*, 1977).

Qualitative phytochemical analysis

The qualitative phytochemical analysis of crude powder of *P. nodiflora* leaf and stem was carried out to identify different phytoconstituents (Harbone, 1998). The phytoconstituents analysed were alkaloids, flavonoids, phenols, saponins, tannins, cardiac glycosides, steroids, phlobatannins, triterpenes, anthocyanins, etc. The presence of specific phytochemicals was indicated with (+) sign and the absence of phytochemicals was indicated with (-) sign. The procedure followed is given in Table 1.

Physicochemical analysis

The physicochemical parameters like loss on drying, total ash, acid-insoluble ash, water-soluble ash, sulphated ash, nitrated ash, carbonated ash and extractive values were determined as per WHO guidelines (WHO, 1998). The solvents used were petroleum ether (PE), toluene (TO), ethyl acetate (EA), methanol (ME) and water (AQ). The details of the procedure are described below:

Loss on drying

Two grams of dried plant powder was taken in a silica crucible. The sample was dried in an oven at 100⁰C-110⁰ C. It was dried until constant weight was obtained. The weight after drying was noted and loss on drying was calculated in mg/g. The percentage was calculated on the basis sample taken initially.

Determination of total ash

Two grams of dried plant powder was taken in a silica crucible. Then it was placed in a Muffle furnace at 500⁰ C for 5 h to remove all the carbon. It was allowed to cool and weighed. Total ash was white in colour which indicated absence of carbon and it was calculated as mg/g of air dried plant material (WHO, 2002).

Determination of acid insoluble ash

25 ml of hydrochloric acid (70g/L) was added to the crucible containing total ash. It was covered with a watch-glass and heated gently for 5 min to boil. The watch-glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ash less filter paper and it was washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible; then it was put in Muffle furnace at 500⁰ C for 2 h. It was allowed to cool and then weighed. Acid insoluble ash was calculated as mg/g of air dried material.

Determination of water soluble ash

25 ml of distilled water was added to the crucible containing total ash and boiled for 5 min. The insoluble matter was collected on an ash less filter paper; then it was washed with hot water and heated in a crucible for 15 min. The weight of insoluble matter was subtracted

from the weight of total ash. The content of water soluble ash was calculated as mg/g of air dried material.

Determination of sulphated ash

Total ash was determined as described above. 2 ml of 0.1 N H₂SO₄ was added to the crucible containing total ash and incubated at room temperature for 10 min; then it was put in a Muffle furnace at 500⁰ C for 5 h. It was allowed to cool and then weighed. The content of sulphated ash was calculated as mg/g air dried material.

Determination of nitrated ash

Total ash was determined as described above. 2 ml of 0.1 N HNO₃ was added to the crucible containing total ash and incubated at room temperature for 10 minutes. Then it was put in Muffle furnace at 500⁰C for 5 h. It was allowed to cool and then weighed. The content of nitrated ash was calculated as mg/g air dried material.

Determination of carbonated ash

Total ash was determined as described above. 2 ml 0.1 N Na₂CO₃ was added to the crucible containing total ash and incubated at room temperature for 10 minutes, then it was put in Muffle furnace at 500⁰C for 5 h. It was allowed to cool and then weighed. The content of carbonated ash was calculated as mg/g air dried material.

Extractive values

The extractive value of dried plant powder was evaluated by extracting the dry powder in solvents of different polarity. The solvents used were petroleum ether (PE), toluene (TO), ethyl acetate (EA), methanol (ME) and water (AQ). The procedure followed was as described below. Five grams of dried plant

powder was taken in 250 ml conical flask and 100 ml of different solvents were added individually and they were placed on a rotary shaker at 120 rpm for 24 h. Then it was filtered by eight layers of muslin cloth and the filtrate was centrifuged at 5000 rpm for 15 minutes. The solvent was evaporated to dryness and dried crude extract was stored in air tight bottles at 4⁰C. It was further used in different analysis.

Fluorescence analysis

Fluorescence study of crude powder of *P. nodiflora* leaf and stem was performed as per kokaski *et al.*, (1958). A small quantity of the crude powder of different plants was placed on a grease free clean microscopic slide and 1-2 drops of freshly prepared various reagent solutions were added, mixed by gentle tilting of the slide. The slides were placed inside the UV chamber and observed in visible light, short (wave length 254 nm) and long (wave length 365 nm) ultra violet radiations. The colors observed by applications of different reagents in different wave lengths were recorded.

Results and Discussion

Organoleptic and macroscopic characteristics

Organoleptic and macroscopic characteristics of *P. nodiflora* leaf and stem are given in Table 2 and Figure 3. The leaf was simple, opposite decussate phyllotaxy, spatulate shape, lobed margin, retuse apex, symmetrical leaf base, scabrous appearance, reticulate venation, odour was characteristic and taste was bitter. The average leaf size was 2.6 -3 cm in length and 1- 1.5 cm in width (Table 2 and Fig. 3). The stem was green, herbaceous, prostrate trailing, cylindrical, up to 36-40 cm in height and 0.2-0.4 in thickness, odour was characteristic, taste was bitter and outer

surface was rough (Table 2).

Microscopic characteristics

The transverse section of *P. nodiflora* is shown in Figure 4. The petiole was kidney shaped. The upper and lower epidermis was single layered (Fig. 4a). Hypodermis was 8-9 celled with collenchymatous tissue. The upper layer was made up of 2-3 parenchymatous cells; vascular bundles were 'arc' shape, the size of the vascular bundles varied from centre to leaf margin that is large too small. They were centripetal arranged i.e. xylem surrounded by the phloem (Fig. 4b). The transverse section of *P. nodiflora* leaf is shown in Figure 4. The leaf lamina was dorsiventral in nature. The upper epidermis and lower epidermis was single layered. The palisade tissue was single layered on the upper surface and was covered with thick cuticle (Fig. 4c). The mesophyll was small 5-7 layered, the ground tissue was made up of parenchymatous cells, and spongy parenchyma cells were present on the lower surface of epidermis (Fig. 4d). T.S. passing through the mid rib region showed vascular bundles towards the ventral surface. The vascular bundles were centrally located conjoint collateral and were surrounded by parenchymatous cells (Fig. 4e). The diacytic stomata were present in lower epidermis; the stomata were surrounded by small subsidiary cells, where as the guard cells were comparatively small in size and each stoma were surrounded by 2 subsidiary cells (Fig. 4f).

The transverse section of *P. nodiflora* stem is shown in Figure 5. The epidermis was single layered with cubical cells surrounded by cuticle (Fig. 5a). The cortex consisted of 4-6 layers, vascular bundles were surrounded by polygonal lignified parenchyma and chollenchyma cells (Fig. 5b). Vascular bundles consisted of secondary phloem with sieve tubes, companion cells and phloem paranchyma; secondary xylem consisted of

metaxylem, lignified trachea, tracheids, few vessels and xylem fibre. Fibers were pitted elongated and moderately thickened (Fig. 5c). Pith was very large and consisted of thin walled sclerenchymatous cells (Fig. 5d).

Powder microscopy

The crude powder of *P. nodiflora* leaf and stem was dark green in colour, fine, odour characteristic and was bitter taste. The powder microscopy characteristics of leaf and stem are shown in Figures 6-7. The specific characteristics determined from the powder study of leaf under microscopic investigation showed unicellular trichomes, diacytic stomata, annular vessles, spiral vessles, etc. The specific characteristics determined from the powder study of stem showed border pitted vessels, annular vessels, spiral vessels, parenchymatous tissue, etc.

Phytochemical analysis

The qualitative phytochemical analysis of crude powder of *P. nodiflora* leaf and stem are given in Table 3. In leaf, alkaloids, flavonoids, phenols and steroids were present in maximum amount followed by quinones; tannins, saponins, cardiac glycosides and coumarins were present in trace amount. Phlobatanins, triterpenes, anthocyanins and Leucoanthocyanins were absent. In stem, alkaloids and flavonoids were present in maximum amount followed by tannins, saponins, phenols and coumarins. Quinones and steroids were present in trace amount while leucoanthocyanin, cardiac glycosides, triterpenes, anthocyanin and phlobatanins were absent

Physicochemical analysis

The physicochemical parameters of the crude powder of *P. nodiflora* leaf and stem of are given in Figure 8. The moisture content of leaf and stem was 8.25 and 9% respectively. The

ash values of leaf and stem are given in Figure 8. The total ash of leaf was 27.41%, water soluble ash was 6.33% and acid insoluble ash was 7%; while for stem they were 12.33%, 4.33% and 2.16% respectively. The sulphated ash, nitrated ash and carbonated ash of leaf were 31.33%, 27% and 27.5% respectively; for stem they were 14.33%, 18.66%, 12.16% respectively. The ash values were more in leaf than in stem.

Extractive values of leaf and stem in different solvents is given in Figure 9. The extractive values for leaf ranged from 0.38% to 7.85% for polar and non-polar solvents while the same for stem ranged from 0.28% to 19.43%. In both leaf and stem, maximum extractive value was in ME solvent and minimum in PE solvent. The water soluble extractive value for leaf and stem were 13.58% and 12.99 % respectively. The non-polar and semi polar solvents had low extractive values while polar solvent methanol and water had maximum extractive values. In ME solvent, the

extractive value in stem was considerably more than in leaf. On the other hand, in aqueous extract, the extractive values of leaf and stem were almost same.

Fluorescence analysis

The fluorescence analysis of crude powder of *P. nodiflora* leaf and stem are shown in Tables 4 and 5. The fluorescence characters of medicinal plants play an important role in the determination of quality and purity of the drug material. In the present study dried powder of medicinal plant *P. nodiflora* was treated with a number of different reagents, which showed characteristic fluorescence at 254 nm and 365 nm wave length. The plant powder of *P. nodiflora* showed different colors at both wave lengths. The colours observed were dark green, green, brown, reddish brown and black for leaf; and dark green, light green, green, yellowish green, whitish green, black, reddish brown and brown for stem.

Table.2 Organoleptic features of *P. nodiflora* leaf and stem

Characters	Observation	Observation
Part	Leaf	Stem
Arrangement	Opposite	Prostrate trailing
Size	2.6- 3 cm long and 1 -1.5 cm wide	36-40 cm long and 0.2-0.4cm wide
Shape	Spathulate	Cylindrical
Color	Green	Green
Odour	Characteristic	Characteristic
Taste	Bitter	Bitter
Appearance	Rough	Rough
Margin	Lobed	-
Apex	Retuse	-
Base	Symmetrical	-
Petiole	Short	-
Texture	Smooth	Rough
Venation	Reticulate	-
Outer surface	Rough	Rough

Table.1 Procedure followed for the estimation various phytochemicals

No.	Phytochemicals	Test	Observation
1	Alkaloids	<p>Add crude powder of leaf and stem to 2N HCl and mixture was filtrated.</p> <p>1) The filtrate was treated with few drops of Dragondroff 's reagent</p> <p>2) The filtrate was treated with few drops of Mayer's reagent</p> <p>3) The filtrate was treated with few drops of Wagner's reagent</p>	<p>Formation of orange precipitate indicated the presence of alkaloids</p> <p>Formation of Cream precipitate indicated the presence of alkaloids</p> <p>Formation of brown precipitate indicated the presence of alkaloids</p>
2	Phenols	The crude powder of leaf and stem was dissolved in distilled water. It was filtered the filtrate was treated with a few drops of 5% FeCl ₃ solution	Formation of deep blue colour indicated the presence of phenols
3	Flavonoids	The crude powder of leaf and stem was dissolved in distilled water. It was filtered the filtrate was treated with a few drops of diluted NaOH, again add few drops of diluted HCl	Formation of yellow orange colour to colourless indicated the presence of flavonoids
4	Saponins (Frothing test)	The crude powder of leaf and stem was vigorously shaken with distilled water and allowed to stand for 10 min	Stable formation of froth for 1 min indicated presence of saponins
5	Tannins (FeCl ₃ test)	The crude powder of leaf and stem was dissolved in distilled water. It was filtered and the filtrate was treated with alcoholic ferric chloride (FeCl ₃) reagent	Formation of blue colour indicated the presence of tannins
6	Steroids (Liebennann – Burchard test)	The crude powder of leaf and stem was dissolved in chloroform. It was filtered and the filtered chloroform extract was treated with acetic anhydride and a few drops of concentrated H ₂ SO ₄	Formation of blue green ring indicated the presence of steroids

7	Phlobatanins	The crude powder of leaf and stem was boiled with 1% aqueous HCl	Formation of red precipitate indicated the presence of phlobatannins
8	Anthocyanins	The crude powder of leaf and stem was dissolved in methanol. It was filtered and the filtered methanolic extract was treated with 2.0 ml NaOH (1 N)	The colour of the solution changed to blue indicated the presence of anthocyanins
9	Triterpenes	The crude powder of leaf and stem was dissolved in chloroform. It was filtered and the filtered chloroform extract was treated with concentrated H ₂ SO ₄	Formation of reddish brown ring indicated the presence of triterpens
10	Cardiac Glycosides (Keller – Kiliani test)	The crude powder of leaf and stem was dissolved in distilled water. It was filtered and the filtered aqueous extract was treated with 1.0 ml mixture of 5 % FeCl ₃ and glacial acetic acid (1.99 V/V). To this solution, few drops of concentrated H ₂ SO ₄ was added	Appearance of greenish blue colour within few minutes indicated the presence of cardiac glycosides
11	Coumarins	The crude powder of leaf and stem was dissolved in distilled water. It was filtered and the filtered aqueous extract was treated with 10 % NaOH	Formation of yellow colour of the solution indicated the presence of coumarins
12	Leucoanthocyanins	The crude powder of leaf and stem was dissolved in distilled water. It was filtered and the filtered aqueous extract was treated with isoamyl alcohol in equal proportion.	Appearance of red colour in upper layer indicated the presence of leucoanthocyanins
13	Quinones	The crude powder of leaf and stem was dissolved in methanol. It was filtered and the filtered methanolic extract was treated with HCl	Formation of yellow precipitation indicated the presence of quinones

Table.3 Qualitative phytochemical analysis of *P. nodiflora* leaf and stem

No.	Phytochemicals	Leaf	Stem
1	Dragondroff's reagent (alkaloids)	+++	+++
2	Mayers test (alkaloids)	+	+

3	Wagner,s test (alkaloids)	++	++
4	Alkaline reagent (flavonoids)	+++	+++
5	Ferric chloride test (tannins)	+	++
6	Phlobatanins	-	-
7	Frothing test (saponins)	+	++
8	Liebennann –Burchard test (steroids)	+++	+
9	Keller – Kiliani test (cardiac glycosides)	+	-
10	Salkowski’s test (triterpenes)	-	-
11	Anthocyanins	-	-
12	5 % Ferric chloride test (phenols)	+++	++
13	Coumarins	+	++
14	Leucoanthocyanins	-	-
15	Quinones	++	+

Note: (+++) more amount, (++) moderate amount, (+) less amount, (-) absent

Table.4 Fluorescence analysis of *P. nodiflora* leaf

No.	Treatment	Under visible light	Under UV light short wave length (254 nm)	Under UV light long wave length (365 nm)
1	1N NaOH (Aq)	Green	Black	Green
2	1N NaOH (Alc)	Green	Green	Brown
3	Ammonia	Green	Black	Dark green
4	Petroleum ether	Green	Black	Dark green
5	50% HCl	Dark green	Black	Black
6	50% H ₂ SO ₄	Dark green	Black	Brown
7	Ethyl-acetate	Dark green	Black	Green
8	Ethyl-alcohol	Green	Black	Green
9	Methanol	Green	Black	Green
10	50%KOH	Green	Black	Black
11	50%HNO ₃	Reddish brown	Black	Black
12	Acetic acid	Green	Black	Green
13	Iodine in water	Green	Black	Dark green
14	FeCl ₃	Dark green	Black	Black

Table.5 Fluorescence analysis of *P. nodiflora* stem

No.	Treatment	Under visible light	Under UV light short wave length (254 nm)	Under UV light long wave length (365 nm)
1	1N NaOH (Aq)	Brown	Black	Brown
2	1N NaOH (Alc)	Brown	Green	Green
3	Ammonia	Brown	Black	Dark green
4	Petroleum ether	Brown	Brown	Light green
5	50%HCl	Light green	Black	Brown
6	50%H ₂ SO ₄	Light green	Black	Greenish brown
7	Ethyl-acetate	Light green	Black	Whitish green
8	Ethyl-alcohol	Green	Black	Whitish green
9	Methanol	Yellowish green	Black	Whitish green
10	50%KOH	Green	Black	Black
11	50%HNO ₃	Reddish brown	Black	Black
12	Acetic acid	Whitish green	Black	Whitish green
13	Iodine in water	Whitish green	Black	Dark green
14	FeCl ₃	Dark green	Black	Black

Fig.1 Different reported activities of *P. nodiflora* L. Greene

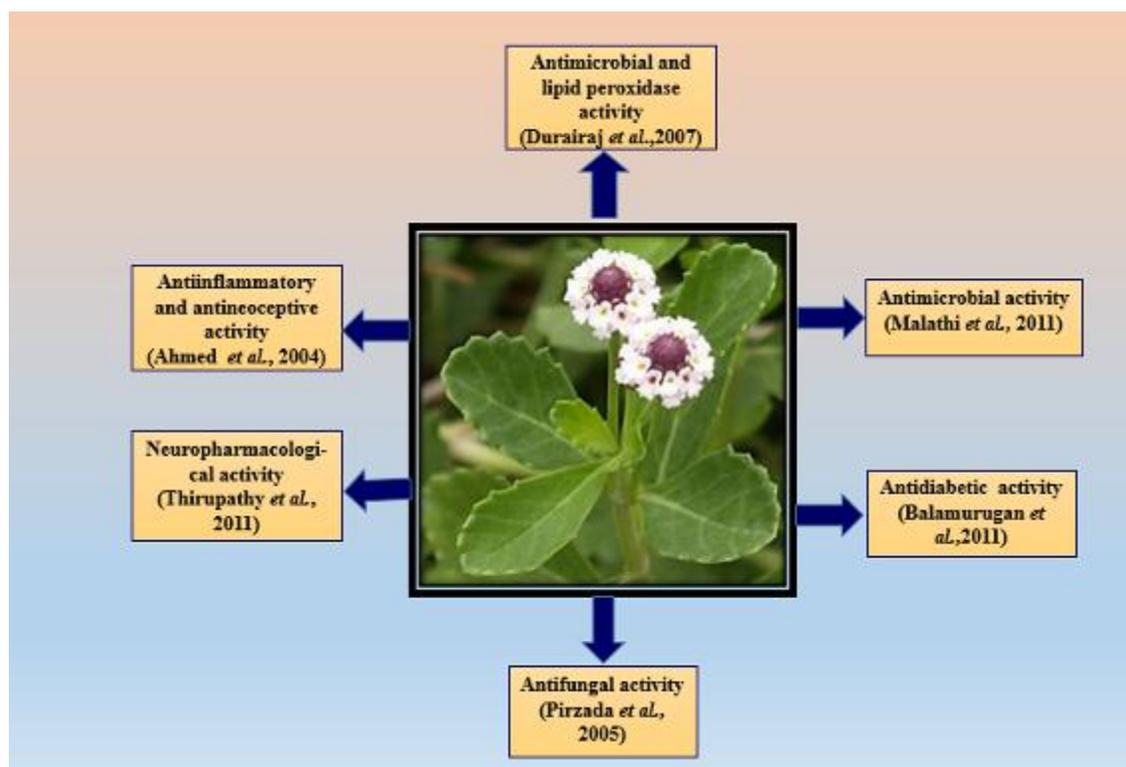


Fig.3 Macroscopic study of *P. nodiflora*

a) Habit



b) Macroscopic study of leaf

c) Macroscopic study of stem



Fig.4 Microscopic study of leaf of *P. nodiflora*



a) T.S of petiole



b) T.S of petiole with vascular bundles



c) T.S of leaf with epidermis



d) T.S of leaf with mesophyll layer



e) T.S of leaf with vascular bundles



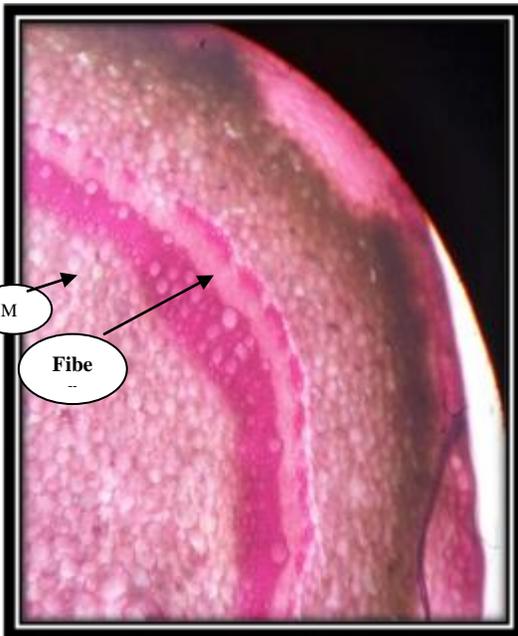
f) Diacytic stomata



a) T.S of stem with epidermis



b) T.S of stem



c) T.S of stem with vascular bundles



d T.S of stem with pith

Fig.6 Photomicrographs of microscopic characteristics of powder of *P. nodiflora* leaf

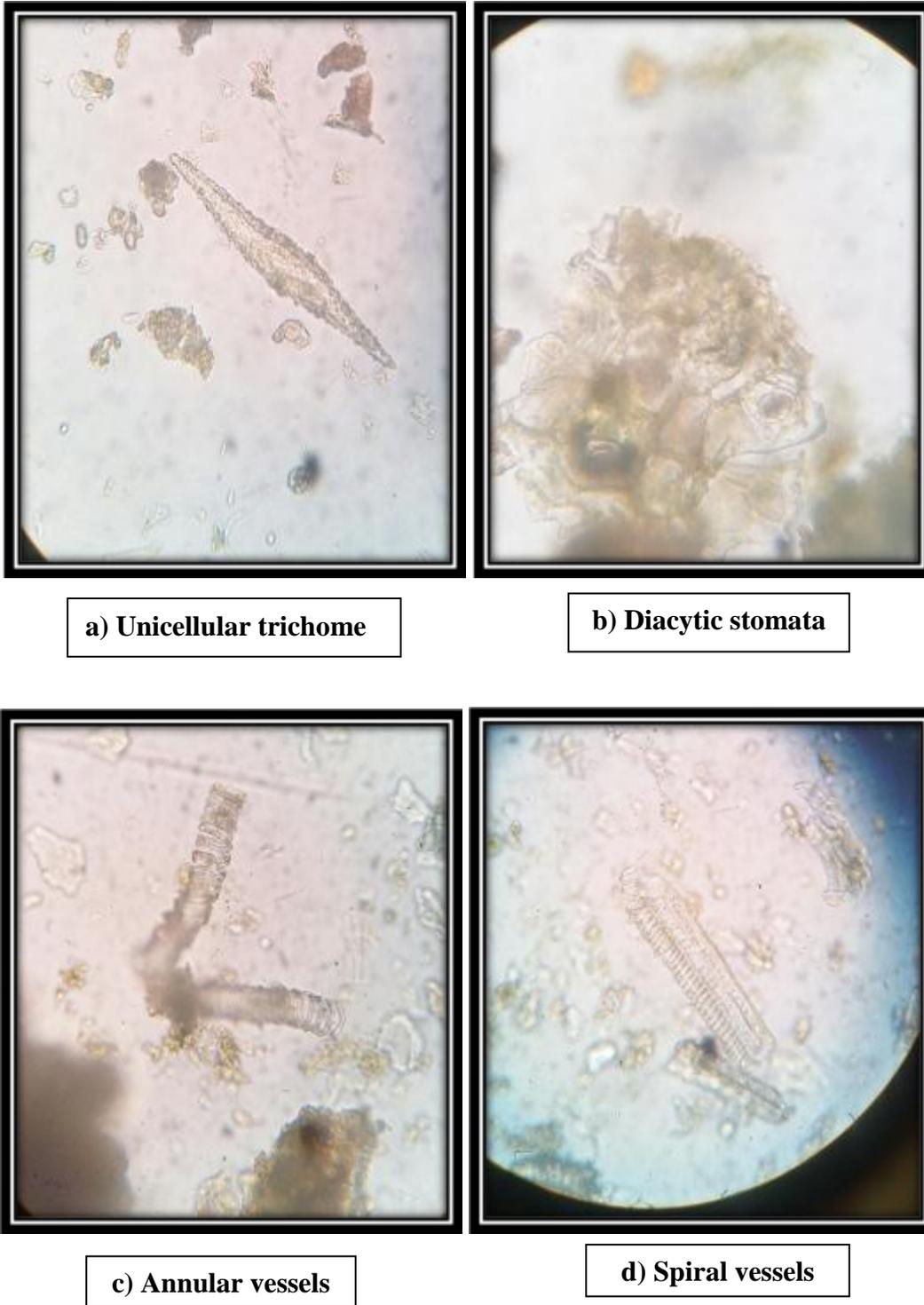
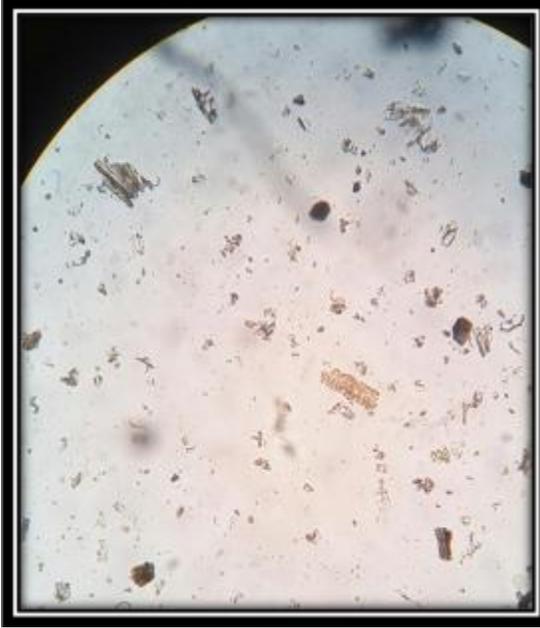


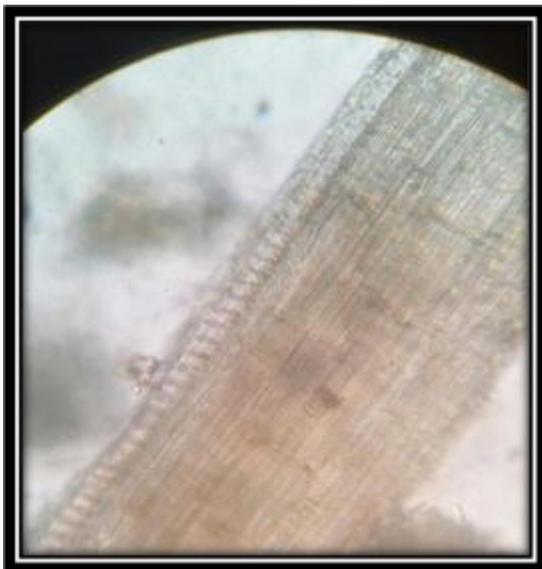
Fig.7 Photomicrographs of microscopic characteristics of powder of *P. nodiflora* stem



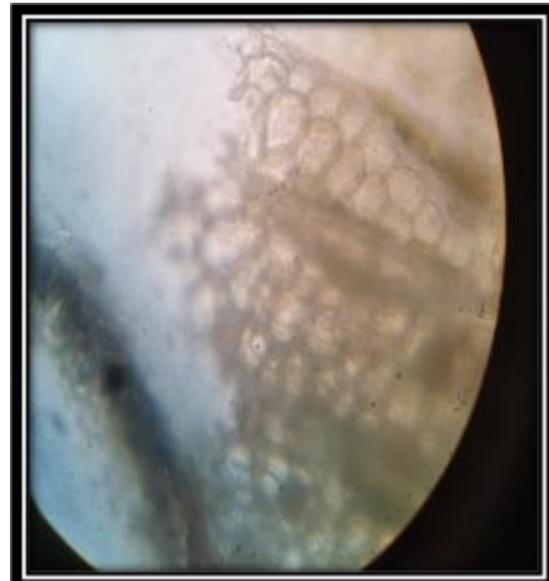
a) Border pitted vessels



b) Annular vessels



C) Spiral vessels



d) Parenchymatous tissue

Fig.8 Ash values of crude powder of *P. nodiflora* leaf and stem

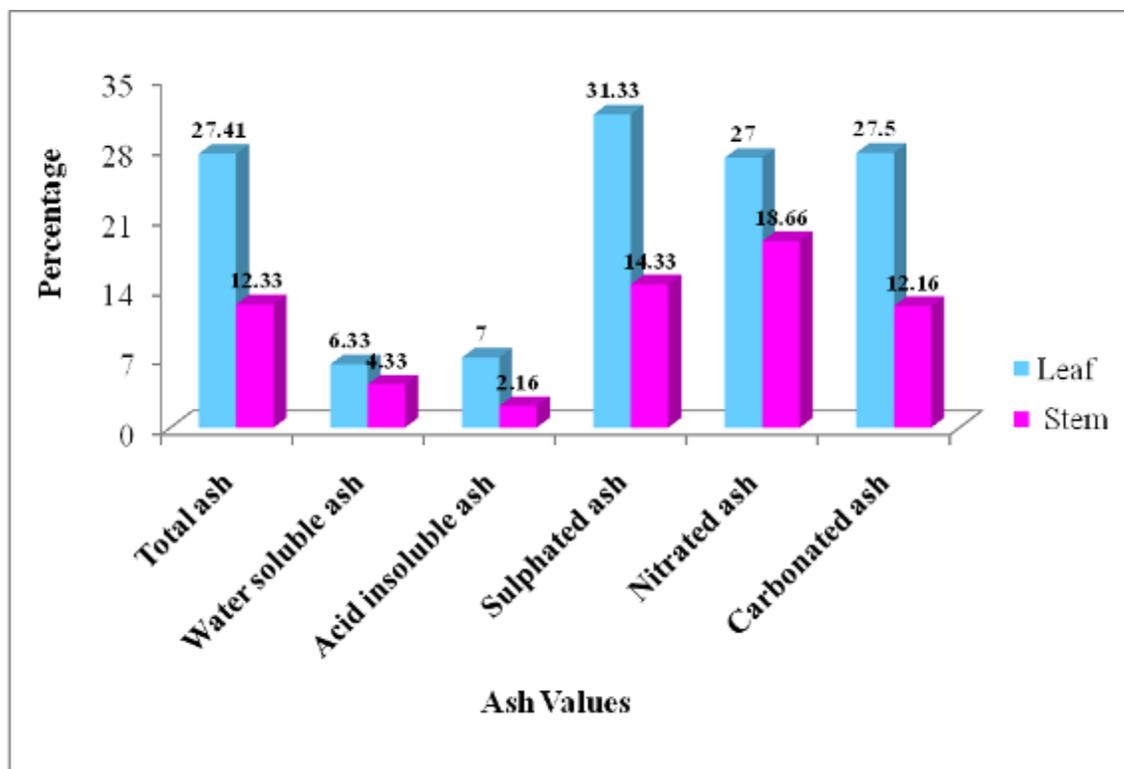


Fig.9 Extractive values of different solvent extracts of *P. nodiflora* leaf and stem

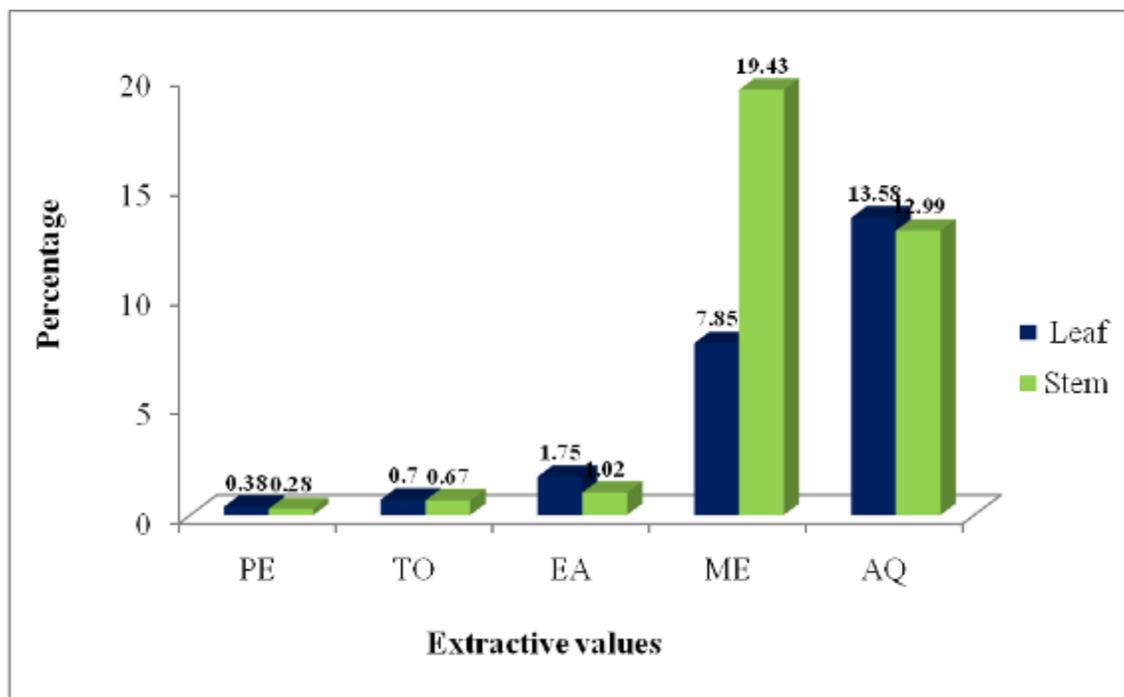
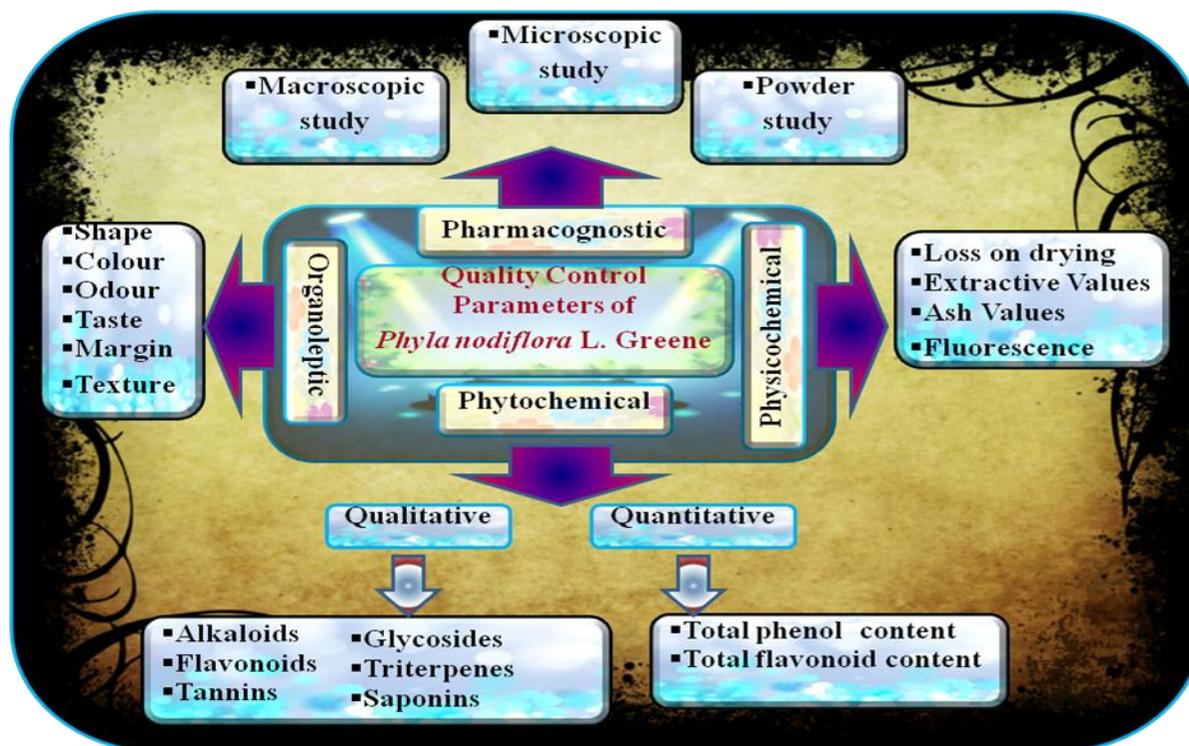


Fig.2 Quality control parameters of *P. nodiflora* L. Greene



Standardization is a necessary and important aspect for the study of identity, purity and quality of crude drug sample of plant origin. There are many methods but easiest, simplest and most popular method is pharmacognostic studies (WHO, 1998). Pharmacognostic studies are reported for other plants like *Madhuka indica* (Moteriya *et al.*, 2015); *Hippeastrum puniceum* (Kurian *et al.*, 2017); *Limonium stocksii* (Kanakiya *et al.*, 2018). Pharmacognostic studies are the first step to establish the identity and the degree of purity of the plant material under study and it will ensure the plant from being adulterated and substituted. In the present study, *P. nodiflora* leaf and stem were evaluated for their pharmacognostic, physicochemical, qualitative and quantitative phytochemical and fluorescence analysis. The macroscopic evaluation showed *P. nodiflora* leaves were green in colour, shape of leaves was spatulate, apex was retuse, base was symmetrical. The microscopic evaluation showed the leaf was dorsiventral in nature, the palisade tissue was on the upper epidermis

and spongy parenchyma cells were present on the lower epidermis. Vascular bundles were towards the ventral surface. They were centrally located, conjoint, collateral; stomata were diacytic. The powder study of leaf showed unicellular trichomes, diacytic stomata, annular vessels and spiral vessels. The macroscopic evaluation showed *P. nodiflora* stem to be green in colour, herbaceous and prostrate trailing. The microscopic evaluation showed the epidermis was single layered, cortex was 4-6 layered, vascular bundles were surrounded by polygonal lignified parenchyma and collenchyma cells; vascular bundles consisted of secondary phloem with sieve tubes, companion cells and phloem parenchyma. The powder study of stem showed the border pitted vessels, annular vessels, spiral vessels and phloem parenchyma. Powder study is very important for authentication of plant when it loses its morphological identity and the drug is in powder form. The diagnostic characters of each plant powder parameters will be helpful for its correct identification

and ensure its purity. It is most simple, easy and cheapest quality control parameter. Some examples of powder study are reported by Menpara and Chanda (2014) for *Pongamia pinnata* L. (Family Fabacea). Sarpong *et al.*, (2017) for *Palisota hirsuta* (Family Commelinaceae), Meghashree *et al.*, (2017) for *Benincasa hispida* (Family Cucurbitaceae).

Phytochemical analysis

Phytochemicals present in the medicinal plants are responsible for the biological activity and it is very important to screen them. The qualitative phytochemical screening was done in crude powder of *P. nodiflora* leaf and stem. In leaf, alkaloids, flavonoids, phenols and steroids were in maximum amount while in stem alkaloids, flavonoids, saponins and phenols were present in maximum amount. Such studies are important since it gives an idea about the phytochemicals present in them and different phytochemical show curative properties to different diseases. Both leaf and stem showed presence of phenols and flavonoids and it is known that plants rich in phenols and flavonoids show good antioxidant activity and plants rich in antioxidant activity show good pharmacological or biological activity (Chanda and Dave, 2009). Similar phytochemical analysis is reported by Tang *et al.*, (2018) for *Psychotria rubra* root, stem and leaves; Kanthale and Biradar (2017) for *Helicteres isora* fruits; *Trianthema portulacastrum* (Pande *et al.*, 2018).

Physicochemical analysis

The physicochemical parameters like loss on drying, total ash, acid insoluble ash, water soluble ash, sulphated ash, nitrated ash, carbonated ash, and extractive values were determined. Loss on drying was 8% in leaf and 9 % in stem. This parameter indicates the efficiency of drying process. It was less than

10% in this plant which is quite less. It indicates that moisture content is less and it will not encourage the growth of bacteria and fungi (Pande *et al.*, 2018). Loss on drying for *Chaetomorpha antennina* was 7% (Dhanki *et al.*, 2018), 9% for *Homalium ceylanicum* (Acharya *et al.*, 2018). The ash values were more in leaf than in stem. The ash values measures the amount of organic and inorganic material present in the plant sample (Shrestha *et al.*, 2014; Yazdinezhad *et al.*, 2016). In *P. nodiflora*, inorganic compounds were present more in leaf than in stem; some of the ash was slightly high as also reported for aerial parts of *Colebrookea oppositifolia* by Ishtiaq *et al.*, (2016).

Extractive values in different solvents give the idea about the nature of secondary metabolites present in the crude drug. In both leaf and stem, the maximum extractive value was in ME solvent and minimum in PE solvent. The water soluble extractive value was also more in both leaf and stem. The results indicate that polar compounds were more than non polar compounds. The non polar compounds were present in trace amount since PE and TO solvent had minimum extractive value both in leaf and stem. Extractive values give an idea about the nature of phytoconstituents present in the crude drug or plant sample. They are also indicative of adulteration so purity of the drug can be checked (Foudah *et al.*, 2015; Netala *et al.*, 2015; Rokad *et al.*, 2018).

Fluorescence analysis

In the present study crude powder of *P. nodiflora* leaf and stem was treated with various reagents which showed characteristic fluorescence at 254 nm and 366 nm wave length. Similar fluorescence analysis is reported for *Terminalia arjuna* (Desai and Chanda, 2014) and for *Zanthoxylum armatum* (Barkatullah *et al.*, 2017). Fluorescence analysis is a necessary parameter for the first

line standardization of the crude drugs. Light reach in short wave length is very active in producing fluorescence and for this reason ultraviolet light is very active in producing fluorescence in many substances which are not visibly fluorescent in visible light.

In conclusion, the quality control parameters i.e. the physicochemical, phytochemical, macroscopic and microscopic characteristic along with the powder studies of leaf and stem of *P. nodiflora* done in the present work are the diagnostic features of *P. nodiflora*. They are useful in maintaining the identity, efficacy and safety of this medicinally useful plant. The phytochemical studies and extractive values will give an idea regarding its use for a particular pharmacological activity.

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