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Pharmacognostic and Preliminary Phytochemical Evaluation of *Centipeda minima* and *Bauhinia purpurea* Leaves

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Abstract

Centipeda minima and Bauhinia purpurea are used as traditional medicinal plant for ailment of various diseases. The present study was aimed to evaluate the Pharmacognostic and Phytochemical screening of Centipeda minima and Bauhinia purpurea. The Pharmacognostic evaluation (total ash, acid insoluble ash, water soluble ash, water soluble extractive and alcohol soluble extractive) and fluorescence analysis of leaves powder were performed. Preliminary phytochemical investigation was carried out of the crude extracts of Centipeda minima and Bauhinia purpurea leaves using solvents of different polarity. The Pharmacognostic characters of Centipeda minima and Bauhinia purpurea have been done for standardization of crude drug. The results of preliminary phytochemical revealed the presence of flavonoids, tannins, phenolic compounds, alkaloids, glycosides, fats and carbohydrates in Centipeda minima and Bauhinia purpurea leaves. These studies provided referential information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario of lack of regulatory laws to control quality of herbal drugs.

1 Introduction

India is the 8th largest country having a total of around 47 thousand plant species, out of which more than 7,500 species are cited as medicinal plants. There are two main reasons for interest in natural products. Firstly the use of medicinal plants as such as phytomedicines, dietary supplements and traditional medicines. Secondly natural products continue to remain an important source of new drug discovery. There are many intrinsic factors which govern the growth and medicinal quality of herbs. This is largely due to change in their chemical constitution which often leads to change in their bioactivity. Due to these inherent uncontrollable variations standardization becomes extremely important. Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. To ensure reproducible quality of herbal products, proper control of starting material is utmost

essential. The first step towards ensuring quality of starting material is authentication^{1,2}.

Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication. Thus, in recent there been a rapid increase standardization of selected medicinal plants of potential therapeutic significance. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and

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the degree of purity of such materials and should be carried out before any tests are undertaken^{3,4}.

Centipeda minima (Asteraceae) is found in moist places. The aerial parts of the Centipeda minima are used to treat headaches, head colds, conjunctivitis, piles and malaria. Previous studies on Centipeda minima exhibited that extract are used for ailment of inflammation, arthritis, analgesic, cancer etc. Phytochemical studies of its composition have led to the identification of a number of flavonoids, polyphenol and terpenes. The chief constituents namely palmitic acid, (Z,Z)-9-,12-octadecatrienoic acid, (Z,Z,Z)-9-,12-octadecatrienoic acid, phytol, naptho[2.3-b]furan-2-(3H)-on, 1-(1,2,3,4,7,7a-hexahydro-1,4,4,5-tetramethyl-1,3a-ethano-3aH-inden-6-yl)etanon, 1,3,5-tri-tertbutyl-benzene, (3Z)-2-methyl-3-octen-2-ol and artemisia ketone present in Centipeda minima^{5,6}.

Bauhinia purpurea belongs to the family Leguminosae, and is a moderate evergreen tree in sub-Himalayan region and western track of India (Khare et al., 2004). Traditionally Bauhinia purpurea are used for the treatment of numerous ailments namely diarrhea, ulcers, enlarge cervical glands, goiter, scrofulous tumors etc. The glycosides, flavonoids, saponins, triterpenoids, phenolic compounds, oxepins, fatty acids and phytosterols secondary metabolites are present in this plant. The two new oxepins named bauhiniastatins1 and 2 isolated from the ethanol extract of whole plant, while root furnishes bauhiniastatins 1, 2, 3 and pacharin^{7,8}. The novel flavone 5,6-dihydroxy-7-methoxyflavone 6-O-b-Dglycoside, xylopyranoside isolated from the chloroform-soluble fraction of the ethanol extract of Bauhinia purpurea stems9. The three different glycerol derivatives and 6-butyl-3-hydroxyflavanone derivatives are 2, 3-dihydroxypropyl oleate, 2,3 dihroxypropyl linoleate, 2,3- dihydroxypropyl 16-hyroxy-decanoate and 6butyl-3-hydroxyflavanone, 6-(3"-oxobutyl)-taxifolin, respectively isolated from methanol extract of heartwood of Bauhinia purpurea10. Bauhinia purpurea extracts was scientifically documented for its antinociceptive, antidiarrhoeal, antiinflammatory, analgesic, anticancer, antipyretic, antimalarial, antimycobacterial, antifungal, anti-diabetics and anti-diarrheal activity etc11-14.

Thus the present investigation was aimed to evaluate the pharmacognostical features and phytochemical analysis of *Centipeda minima* and *Bauhinia purpurea* for identification and authentication of the plant.

2 Materials and Methods

2.1 Plant material

The leaves of *Centipeda minima* and *Bauhinia purpurea* were selected for the proposed study. The plant material was authenticated by Dr. A.P. Singh, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). The leaves were shade dried,

reduced to coarse powder and stored in airtight container till further use.

2.2 Pharmacognostic evaluation

Air-dried powdered material was subjected to qualitative and quantitative physicochemical estimations. The procedures were followed as mentioned earlier.

2.2.1 Physical evaluation

The physical values like total ash, acid insoluble ash, water-soluble ash, alcohol soluble extractive and water-soluble extractives were determined.

2.2.1.1 Ash values

a) Determination of total ash value

Accurately weighed about 3 gms of air dried powdered drug was taken in a tared silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air dried drug.

b) Determination of acid insoluble ash value

The ash obtained as directed under total ash was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

c) Determination of water soluble ash value

The total ash obtained was boiled with 25 ml. of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

2.2.1.2 Loss on drying

Loss on drying is the loss in weight in % w/w determined by means of the procedure given below. It determines the amount of volatile matter of any kind (including water) that can be driven off under the condition specified (Dessicator or hot air oven). If the sample in the form of large crystals, then reduce the size by quickly crushing to a powder.

About 1.5 gm. of powdered drug was weighed accurately in a tared porcelein dish which was previously dried at 105°C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated.

2.2.1.3 Extractive values

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

a) Determination of alcohol soluble extractive value

5 gm of the air-dried coarse powder of the plant material was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105 °C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air-dried drug.

b) Determination of water soluble extractive value

Weigh accurately the 5 gm of coarsely powdered drug and macerate it with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug ¹⁵⁻¹⁷.

2.2.1.4 Fluorescence analysis

Fluorescence characteristics of the powdered plant material of *Centipeda minima* and *Bauhinia purpurea* were observed in daylight and UV light. Also the fluorescent study was performed on treating the drug powder with different chemical reagents ¹⁸⁻

2.3 Preliminary phytochemical studies

2.3.1 Preparation of extract

The powdered leaves of *Centipeda minima* and *Bauhinia* purpurea about 1 Kilogram were packed separately in soxhlet apparatus. Further extracted with petroleum ether, hydroalcohol (mixture of 70% ethanol and 30% distilled water) and distilled water separately, until the completion of the extraction. The extract was filtered while hot, and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely, and later dried in a desiccator.

2.3.2 Qualitative chemical tests

Qualitative chemical tests were performed to determine the presence of alkaloids, carbohydrates, cardiac glycosides, polyphenols, saponins, tannins and terpenoids.

2.3.2.1 Test for alkaloids

- (a) Dragendorff's test: To 1 ml of the extract, add 1 ml of dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.
- (b) Mayer's test: To 1 ml of the extract, add 1 ml of mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.
- (c) Hager's test: To 1 ml of the extract, add 3ml of Hager's reagent (Saturated aqueous solution of picric acid), yellow coloured precipitate indicates the presence of alkaloids.
- (d) Wagner's test: To 1 ml of the extract, add 2 ml of wagner's reagent (lodine in Potassium lodide), Formation of reddish brown precipitate indicates the presence of alkaloids.

2.3.2.2 Test for Proteins

- (a) Biuret test: Added 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color was produced, and then added to the 1ml of the extract. Formation of pinkish or purple violet color indicated the presence of proteins.
- (b) Ninhydrin test: Added two drops of freshly prepared 0.2% Ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heated. Development of blue color revealed the presence of proteins, peptides or amino acids.
- (c) Millon's test: 1ml of test solution was made acidify with sulphuric acid and added Millon's reagent and boiled this solution. A yellow precipitate was formed indicated the presence of protein.
- (d) Xanthoproteic test: To 1ml of the extract, added 1ml of concentrated nitric acid. A white precipitate was formed, it is boiled and cooled. Then 20% of sodium hydroxide or ammonia was added. No formation of orange color indicated the absence of aromatic amino acids.

2.3.2.3 Test for glycosides

- (a) Legal test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.
- (b) Baljet test: To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.
- (c) Keller-Killiani test: 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of

lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer is separated in a porcelein dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

(d) Borntrager's test: Add a few ml of dilute Sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of red colour of the ammonical layer shows the presence of anthraquinone glycosides.

2.3.2.4 Test for carbohydrates and sugars

- (a) Molisch's test: To 2ml of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.
- (b) Fehling's test: To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars
- (c) Benedict's test: To 5ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

2.3.2.5 Test for tannins and phenolic compounds

- (a) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.
- (b) To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins.
- (c) The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.

2.3.2.6 Test for flavonoids

- (a) The drug in alcoholic and aqueous solution with few ml of ammonia is seen in U.V. and visible light, formation of fluorescence indicates the presence of flavonoids.
- (b) Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow colour

- solution formed, disappears on addition of an acid indicates the presence of flavonoids.
- (c) Shinoda's test: The alcoholic extract of powder treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonols.
- (d) The extract is treated with sodium hydroxide, formation of yellow colour indicates the presence of flavones.
- (e) The extract is treated with concentrated H_2SO_4 , formation of yellow or orange colour indicates flavones.

2.3.2.7 Test for steroids

- (a) Libermann-Burchard test: 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.
- (b) Salkowski test: Dissolve the extract in chloroform and add equal volume of conc. H₂SO₄. Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

2.3.2.8 Test for fixed oils and fats

- (a) Spot Test: Press a small quantity of extracts between the filter paper. Oil stains on paper indicates the presence of fixed oils.
- (b) Saponification test: To 1ml of the extract, add few drops of 0.5 N alcoholic Potassium hydroxide along with a drop of phenolphthalein. Heat the mixture on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats²⁰⁻²³.

3 Results and Discussions

3.1 Pharmacognostical studies

The safety and efficacy of herbal products are dependent upon the standardization of the herbs used¹⁻³. The physicochemical characters of all plant have been done for standardization of crude drug. The physicochemical characters of *Bauhinia purpurea* and *Centipeda minim*were studied, and the results are presented in tables 1 and 2, respectively.

The ash value is used for the determination of inorganic materials, such as carbonate, silicates, oxalates, and phosphates. Heating causes the loss of organic material in the form of CO_2 leaving behind the inorganic components. The value obtained for the *Bauhinia purpurea* and *Centipeda minim* were around 6.4% and 5.2%, respectively as total ash (Table 1).

The acid insoluble ash determines the acid insoluble material present in the drug material and the water soluble ash determines the water soluble material specifically the water soluble inorganic salts. The acid insoluble values of *Bauhinia purpurea* and *Centipeda minim* were 1.5% and 1.1%, respectively. The water soluble ash values of *Bauhinia purpurea* and *Centipeda minim* were 2.2% and 3.1% respectively. The loss on drying of the *Bauhinia purpurea* and *Centipeda minim* powder were 13.4% and 11.7%, respectively (Table 1).

The alcohol extractive values of *Bauhinia purpurea* and *Centipeda minim* were 20.3% and 16.4%, respectively. The water extractive values of *Bauhinia purpurea* and *Centipeda minim* were 28.5% and 26.7%, respectively (Table 1). The findings of extractive values indicate that both plants contains higher amount of highly water soluble phytoconstituents.

The fluorescence character of powdered drug plays a vital role in the determination of quality and purity of the drug material.

Table 1: Physical parameters of plant powder of *Bauhinia* purpurea and *Centipeda minima*

	Observations (% w/w)			
Studied parameters	Bauhinia purpurea	Centipeda minima		
Loss on drying	13.4±0.45	11.7±0.61		
Total ash value	6.4±0.05	5.2±0.08		
Acid insoluble ash value	1.5±0.02	1.1±0.05		
Water soluble ash value	2.2±0.07	3.1±0.07		
Alcohol extractive value	20.3±0.19	16.4±0.03		
Water extractive value	28.5±0.20	26.7±0.29		

Values are mean ± SEM of three determinations

Table 2: Fluorescence analysis of Bauhinia purpurea and Centipeda minim powder

Chemical Treatment	Day	light	UV Light		
	Bauhinia purpurea	Centipeda minima	Bauhinia purpurea	Centipeda minima	
Powder as such	Green	Green	Green	Green	
Powder + 1 N HCl	Colourless	Colourless	Blue Green	Green	
Powder + aqueous 1 N NaOH	Yellowish Green	Yellowish Green	Dark Green	Fluorescence Yellowish Green	
Powder + alcoholic 1 N NaOH	Green	Green	Dark Green	Fluorescence Green	
Powder + 50% HNO ₃	Yellowish orange	Light Yellow	Dark Green	Fluorescence Yellowish Green	
Powder + 50% H ₂ SO ₄	Yellowish Green	Light Yellow	Green	Green	
Powder + Methanol	Green	Green	Green	Greenish Brown	
Powder + Water	Light Yellow	Green	Yellowish Green	Green	

The powder drugs exhibit different fluorescence character in the presence of different chemical reagents under ultra-violet light due to presence of different functional groups in drug. The results of fluorescence characteristics of plant are displayed in table 2. The fluorescence analysis is a tool for the qualitative analysis of crude drug.

3.2 Extraction of plant material

Presence of classes of secondary metabolite may be a useful indicator of both efficacy and potential toxicity; hence test for the presence of phytochemical classes with known bioactivity was done. The yield of petroleum extracts of leaves of *Bauhinia purpurea* and *Centipeda minima* were 2.5% and 1.8%, respectively. The yield of hydroalcoholic extracts of leaves of

Bauhinia purpurea and Centipeda minima were 21.3% and 17.8%, respectively. While the yield of aqueous extracts leaves of Bauhinia purpurea and Centipeda minima were found to be 29.6% and 28.2%, respectively (Table 3).

The study revealed that the leaves of *Bauhinia purpurea* and *Centipeda minima* contain higher amount of semi polar and polar secondary metabolites. The pharmacological activity of plant material varies according to its polarity or nature of phytoconstituents. Further phytochemical screening of all the extracts obtained from leaves of *Bauhinia purpurea* and *Centipeda minima* were performed.

3.3 Phytochemical screening

The plant may be considered as a biosynthetic laboratory, not only for the chemical compounds such as Carbohydrates, Protein and Lipids that are utilized as food by men, but also for a multitude of compounds like Glycosides, Alkaloids, Volatile oils, Tannins etc., that exerts a physiologic effect. The compounds that are responsible for therapeutic effect are usually the secondary metabolites. A systemic study of a crude drug embraces through consideration of both primary and secondary metabolites derived as a result of plant metabolism.

The plant material may be subjected to preliminary phytochemical screening for the detection of various plant constituents²⁴.

3.3.1 Phytochemical screening of Bauhinia purpurea

Preliminary phytochemical investigations of the extracts of leaves of *Bauhinia purpurea* revealed the presence of flavonoids, tannins, phenolic compounds, alkaloids, glycosides, fats and carbohydrates. The details are presented in table 4.

Table 3: Characteristics of various extract of Bauhinia purpurea and Centipeda minima

Extract	Yield ((% w/w of powdered	wdered drug) Physical appearance)
	Pet. Ether	Hydroalcoholic	Aqueous	Pet. Ether	Hydroalcoholic	Aqueous
Bauhinia purpurea	2.5	21.3	29.6	Semisolid	Solid	Solid
Centipeda minima	1.8	17.8	28.2	Semisolid	Solid	Solid

Table 4: Phytochemicals present in leaves of Bauhinia purpurea extracts

	Phytoconstituent	Pet. Ether	Hydroalcoholic	Aqueous
	Dragendorff's test	-	-	-
	Hager's test	-	+	-
Alkaloids	Mayers	-	+	-
	Wagners	-	+	-
	Legal's test	-	+	+
	Keller killiani test	-	+	+
Glycosides	Baljet test	-	+	-
	Keller-Killiani test	-	-	-
	Borntrager's test	-	+	+
	Molish test	-	+	+
Carbohydrates	Benedict's test	-	+	+
	Fehling's test	-	+	-
	5%Fecl ₃ solution	-	+	+
Tannins and	Lead acetate solution	-	+	+
Phenolic	Bromine water	-	+	-
compound	Potassium ferric cyanide and ammonia solution	-	+	+
Flavonoids	Shinoda test	-	+	+
C+:	Liebermann burchard test	-	-	-
Steroid test	Salkowski test	-	-	-
Dratain	Biuret test	-	-	-
Protein	Ninhydrin test	-	-	-
Fat and all to a	Saponification test	-	-	-
Fat and oil test	Spot Test	+	-	-

^{+ =} Present, - = Absent

From the result of phytochemical screening, the petroleum ether extract of leaves of *Bauhinia purpurea* exhibited the presence of fats and oils. Alkaloids, glycosides, carbohydrates, flavonoids, tannins and polyphenol were found in hydroalcoholic extracts of leaves of *Bauhinia purpurea*. Similarly glycosides, carbohydrates, flavonoids, tannins and polyphenol were existing in aqueous extracts of leaves of *Bauhinia purpurea*. The maximum phytocostituents were observed in hydroalcoholic extracts of leaves of *Bauhinia purpurea* (Table 4). Now

hydroalcoholic and aqueous extracts of *Bauhinia purpurea* were selected for further *in vitro* antioxidant activity evaluation as this extract revealed the presence of flavonoids and phenolic compounds.

3.3.2 Phytochemical screening of Centipeda minima

Preliminary phytochemical investigations of the extracts of leaves of *Centipeda minima* revealed the presence of flavonoids, tannins, phenolic compounds, alkaloids, glycosides, fats and carbohydrates. The details are presented in table 5.

Table 5: Phytochemicals present in leaves of Centipeda minima extracts

Ph	ytoconstituent	Pet. Ether	Hydroalcoholic	Aqueous
	Dragendorff's test	-	+	-
Alkaloids	Hager's test	-	+	-
	Mayers	-	-	-
	Wagners	-	-	-
	Legal's test	-	+	+
Observation	Keller killiani test	-	+	+
Glycosides	Saponin glycoside	-	-	-
	Coumarin glycoside	-	-	-
	Molish test	-	+	+
Carbohydrates	Benedict's test	-	+	+
	Tannic acid test for starch	-	-	+
	5%Fecl ₃ solution	-	+	+
	Lead acetate solution	-	+	-
Tannins and Phenolic	Bromine water	-	-	-
compound	Acetic acid solution	-	+	+
	Dilute potassium permanganate solution	-	-	-
Flavonoids	Shinoda test	-	+	+
Otanaid taat	Liebermann burchard test	-	-	-
Steroid test	Liebermann's reaction	-	-	-
Protein	Biuret test	-	-	-
	Ninhydrin test	-	-	-
Fot and all to -t	Solubility Test	++	-	-
Fat and oil test	Filter paper staining	+	-	-

^{+ =} Present, - = Absent

From the result of phytochemical screening, the petroleum ether extract of leaves of *Centipeda minima* demonstrated the presence of fats and oils. Alkaloids, glycosides, carbohydrates, flavonoids, tannins and polyphenol were found in hydroalcoholic extracts of leaves of *Centipeda minima*. Similarly glycosides, carbohydrates, flavonoids, tannins and polyphenol were present in aqueous extracts of leaves of *Centipeda minima*. The maximum phytocostituents were observed in hydroalcoholic extracts of leaves of *Centipeda minima* (Table 5). Now hydroalcoholic and aqueous extracts of *Centipeda minima* were

selected for further *in vitro* antioxidant activity evaluation as this extract revealed the presence of flavonoids and phenolic compounds.

4 Conclusion

The present study on pharmacognostic and phytochemical investigation of *Centipeda minima* and *Bauhinia purpurea* leaves will be providing useful information in regard to its correct identity and help to differentiate from the closely related other species of *Centipeda minima* and *Bauhinia purpurea*.

5 Conflicts of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

6 Author's contributions

AS and VDT carried out literature review and experimental work of the present study. RKS and EMF carried out discussion of the present study. All authors read and approved the final manuscript.

7 References

- Pandey MM, Rastogi S, Rawat AKS. Indian herbal drug for general healthcare: an overview. The Internet Journal of Alternative Medicine. 2008; 6(1): 1-3.
- Reddy YSR, Venkatesh S, Ravichandran T, Subbaraju T, Suresh B. Pharmacognostical studies of Wrightia tinctoria bark. Pharma Biol 1999:37: 291-5.
- Venkatesh S, Madhava RB, Suresh B, Swamy MM, Ramesh M. Pharmacognostical identification of Rumex nepallensis Spreng (Polygonanceae)- An adulterant for Indian Rhubarb. Nat Prod Sci 2004;10: 43-7.
- World Health Organization. Quality control methods for medicinal plant materials. Geneva: WHO Library; 1998: 1-115.
- Hengxing Liang, Fukai Bao, Xiaoping Dong, Rui Tan, Caijun Zhang, Qing Lu, Yongxian Cheng. Antibacterial Thymol Derivatives Isolated from Centipeda minima. Molecules. 2007; 12: 1606-1613.
- Liu ZG, Yu HM, Wen SL, Liu YL. Histopathological study on allergic rhinitis treated with *Centipeda minima*. Zhongguo Zhong Yao Za Zhi. 2005; 30(4): 292-4.
- Kumar, T., Chandrashekar, K.S., 2011. Bauhinia purpurea Linn.: A Review of its Ethnobotany, Phytochemical and Pharmacological Profile. Research Journal of Medicinal Plants, 5, 420-431.
- Pettit, G.R., Numata, A., Iwamoto, C., Usami, Y., Yamada, T., Ohishi, H., Cragg, G.M., 2006. Antineoplastic agent 551 isolation and structure of Bauhiniastatins-1-4 from *Bauhinia purpurea*. J Nat. Prod. 69, 323-327.
- Yadav, R.N., Tripathi, P., 2000. A novel flavone glycoside from the stem of *Bauhinia purpurea*. Fitoterapia. 71, 88-90.
- Kuo, Y.H., Yeh M.H., Huan, S.L., 1998. A novel 6- butyl-3-hydroxyflavanone from heartwood of *Bauhinia* purpurea. Phytochemistry. 49: 2529-2530.

- Marimuthu, K., Dhanalakshmi, R., 2014. A Study on Phytochemicals in *Bauhinia purpurea* L. Leaf and Flower. Int. J. Pharm. Sci. Rev. Res. 29(2), 72-76.
- Muralikrishna, K.S., Latha, K.P., Shreedhara, C.S., Vaidya V.P., Krupanidhi, A.M., 2008. Effect of *Bauhinia* purpurea Linn. on alloxan-induced diabetic rats and isolated frogs heart. Int. J. Green Pharm. 2, 83-86.
- Boonphong, S., Puangsombat, P., Baramee, A., Mahidol C., Ruchirawat S., Kittakoop, P., 2007. Bioactive compounds from *Bauhinia purpurea* possessing antimalarial, antimycobacterial, antifungal, antiinflammatory and cytotoxic activities. J. Nat. Prod., 70, 795-801.
- Zakaria, Z.A., Rahman, N.I.A., Loo, Y.W., Ayub, A.H.A., Sulaiman M.R., 2009. Antinociceptive and antiinflammatory activities of the chloroform extract of *Bauhinia purpurea* L. (Leguminosae) leaves in animal models. Int. J. Trop. Med., 1, 140-145.
- Mukherjee PK. Quality Control Herbal Drugs–An approach to evaluation of botanicals. Business Horizons Pharmaceutical Publishers. New Delhi. 2005; 131-196.
- Harbone JB. Phytochemical Methods-a guide to modern techniques of plant analysis. 3rd ed. Springer, New Delhi. 2005; 1-32.
- Kandelwal KR, Pawar AP, Kokate CK, Gokhale SB. Practical Pharmacognosy Technique and Experiments, 3rd edition, Nirali Prakashan. 1996; 2-4.
- Pratt RT, Chase CR. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. Journal of American Pharmaceutical Association. 1949; 38: 324-331.
- Kokoshi CJ, Kokoshi RJ, Sharma FT. Fluorescence of powdered vegetable drugs under ultra-violet radiation, Journal of American Pharmaceutical Association. 1958; 47: 715.
- Singh B, Ahamad A, Pal V. Evaluation of Antibacterial Activity and Phytochemical Screening of Azadirachta indica Leaves Extracts Against Staphylococcus aureus. UK Journal of Pharmaceutical and Biosciences. 2015; 3(4): 43-47.
- 21. Gupta AK, Ahirwar NK, Shinde N, Choudhary M, Rajput YS, Singh A. Phytochemical Screening and Antimicrobial Assessment of Leaves of Adhatoda vasica, Azadirachta indica and Datura stramonium. UK Journal of Pharmaceutical and Biosciences. 2013; 1(1): 42-47.

- 22. Purohit P, Bais RT, Singh P, Khan S. Assessment of antibacterial activity and phytochemical screening of Hemidesmus indicus root extracts. UK Journal of Pharmaceutical and Biosciences. 2014; 2(6): 67-72.
- 23. Kenwat R, Prasad P, Sahu RK, Roy A, Saraf S. Preliminary phytochemical screening and in vitro antioxidant efficacy of fruit oil of Martynia annua. UK
- Journal of Pharmaceutical and Biosciences. 2014; 2(1): 16-22.
- 24. Kumar M, Mondal P, Borah S, Mahato K. Physicochemical evaluation, preliminary phytochemical investigation, fluorescence and TLC analysis of leaves of the plant Lasia spinosa (Lour) Thwaites. Int J Pharm Pharm Sci. 2013; 5(2): 306-310.