Ethnobotanical Leaflets 12: 461-468. 2008.

Studies on some Pharmacognostic Profiles of *Bauhinia purpurea* Linn. Leaves (Caesalpinaceae)

M. Sugumaran* and T. Vetrichelvan

Department of Pharmaceutical chemistry, Adhiparasakthi College of Pharmacy,
Melmaruvathur – 603 319, Tamil Nadu.

*E-mail: murugesansugumaran@yahoo.com

Issued 2 July 2008

Abstract

The leaves of *Bauhinia purpurea* Linn. were studied with the aim of determining the following pharmacognostical parameters for this species: Macroscopical characters; Leaf constants; Physico-chemical constants; Extractive values; Colour; Consistency; Extractive values with different solvents; Micro chemical tests; Fluorescence characters of liquid extracts and leaf powder after treatment with different chemical reagents under visible and UV light at 254nm&366 nm; Measurement of cells and tissues; Bulk density angle of repose; and, Powder microscopy. Hopefully, the determination of these characteristics will aid future investigators in their pharmacological analyses of this species. Preliminary pytochemical studies on different extracts of the leaves were also performed.

Introduction

Bauhinia purpurea Linn. (Caesalpinaceae) is an ornamental plant found throughout subtropical, India, North and South America, Nepal, Australia, Africa and United Kingdom. The plant is commonly known as Mandarai in Tamil and khairwal in Hindi[1]. The flavone glycoside, 5,6-dihydroxy-7-methoxy flavone 6-O-beta-D-xylopyranoside, was isolated from the chloroform-soluble fraction of the ethanolic extract of *bauhinia purpurea* stems, and a new flavone glycoside, 6,4' dihydroxy 3- prenyl 3,5,7,5' tetra methoxy flavone-6-O-a-L-rhamnopyranoside, has been found in the seed of *bauhinia purpurea* [2, 3].

Numerous types of biological activities are attributed to bauhinia species. B. *purpurea* is the most important species used to treat many ailments in traditional system of medicine. (Table 1).[4-7] It is also reported for its antidiarrhoeal , anticancer and thyroid gland stimulating properties.[8-10] Hence, country traders often subject it to adulteration/ substitution .

Table 1. Ethnomedical information for *bauhinia purpurea* Linn.

Parts	**Uses**
Flowers	Laxative
Root	Carminative

Root bark	Mixed with curd and used in hemorrhoids. Its paste with dried ginger applied internally in the treatment of goiter.
Stem bark	Astringent in diarrhea
Bark	Decoction is used as a wash in ulcer
Flower buds	Eaten as a vegetable, laxative, anthelmintic , useful in piles and blood dysentery
Others	Dropsy, anasarca, pain, rheumatic thigh swelling, deer-epilepsy, convulsion, delirium febris, animal bite, datura intoxication and anti thyroid

-

But, no pharmacognostical work has been done on a drug plant of such potential until the present time. Therefore, the aim of the present investigation has been to study the important pharmacognostical characteristics of the leaves of *bauhinia purpurea* in both whole and powdered form.

Materials and Methods

Plant Material

The plant material was collected from the village of Melmaruvathur (District Kancheepuram) in the month of January 2007. The plant was identified by local people of that village and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Chennai. A herbarium specimen of the plant (MS–4) was preserved in the Department of pharmacognosy of our Institute for further reference. The leaves were separated and dried under shade, pulverized by mechanical grinder, passed through 40 mesh sieve and stored in a closed vessel for further use. All the reagents used were of analytical grade obtained from S.D. Fine chemicals Ltd., Mumbai and Qualigens fine chemicals, Mumbai.

Methods

The macroscopical characters (size, shape, colour, odour, taste, surface, texture, venation, margin, base, and petiole) of the leaves were observed[11]. Then, for powder microscopical study, the powder was stained with phloroglucinol and concentrated HCl to study the lignified cells, lignified parenchyma, trichomes, fibres, xylem vessels, mesophyll, palisade cells and stomata, etc. The powder was also stained with N/50 iodine solution to detect the presence of starch. A small portion of powder was mounted in water to identify calcium oxalate crystals[11]. Quantitative microscopy was determined by methods prescribed by Trease and Evans[12].

The ash values , alcohol soluble and water soluble extractives values and Loss on drying of leaves were determined as per the Indian Pharmacopoeia methods.[13] The crude fiber content was done by Dutch process.[14] The behaviour of the powdered leaves with different chemical reagents was studied.[15] The fluorescence characters of the various extracts and powdered leaf with different chemical reagents were observed under day light and UV light

(254nm&366nm), by following procedure reported by Kokoshi et al.[16] Measurements of the cells/ tissues were made with the help of micrometer under a compound microscope.[17] Other extractive values were determined successively starting from petroleum ether (60-80°), chloroform, acetone, ethylacetate, methanol and distilled water by using soxhlet extraction apparatus.[18] For this purpose the powder (100g) was successively hot extracted with 300ml of above solvents for 72 h. Before switching over to the next solvent, the powder under extraction (marc) was dried to remove the traces of earlier solvent. The dried extractives were obtained after evaporation of solvent under reduced pressure.

The angle of repose of powder was determined by the funnel method. The accurately weighed powder was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of the powders. The powder was allowed to flow through the funnel freely onto the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation, Tan q = h/r, Where h and r are the height and radius of the powder cone.[19]

For the determination of bulk density, 2 g of powder, previously lightly shaken to break any agglomerates formed, was introduced into a 10 ml, measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall under its own weight onto a hard surface from the height of 2.5 cm at 2 seconds intervals. The tapping was continued until no further change in volume was noted. Bulk density was calculated for dried powdered drug using following formula: Bulk Density = Mass of Powder/ Bulk volume.[20]

Preliminary phytochemical tests of different extracts were performed by using specific reagents through standard procedures.[21-25]

Results

Analysis and Discussion

Colour: Green; Odour: Odourless; Size:8-15 cm in diameter&10-20cm long; Shape: Shallowly cordate; Taste: Slightly bitter; Surface: Glabrous; Texture: Coriaceous; Apex: marginate ; Margin: Sinuate; Venation: Parallel ; Petiole : Size: 4-4.5 cm ; Base: Stipulate.

The physical constant values of total ash (7.05%), acid insoluble ash (2.72%), water insoluble ash (2.98%), sulphated ash (7.91%), loss on drying (12.87%), crude fiber content (27.76%) of leaves which are specific to the plant identification. The methanol and water soluble extractive values were 35.52% and 24.4% respectively, which indicated the nature of constituents present. Quantitative microscopical study also give valuable information regarding specific leaf constants such as vein islet no (15) , vein termination no (65), palisade ratio (78), stomatal no (3), and stomatal index (20), etc. Fluorescence studies on extracts revealed different shades of green fluorescence under UV light at 254 nm. The size of the cell elements like trichomes (66.5 -117.04 -159.6 ´ 13.3 -14.3 -26.3m), starch grains (6.65-14.63-26.6m), fibers (399 -687.8-997.5 ´ 26.3-41.2-99.3) and xylem vessels (93.1 -133.4 -226.1´ 26.3 - 46.7 - 67.5 70.8m) were recorded.

Powder of P. *dulce* is pale green, fine and tasteless but with a characteristic odour. Other features of the powder were the presence of covering trichomes, xylem vessels, paracytic stomata, calcium oxalate crystals in the form of sheeth and starch grains, etc. The behaviour of leaf powder (Table 2) upon treatment with different chemical reagents was also studied.

Table 2. *Behavior of powdered leaves on treatment with different chemical reagents.*

Reagents	**Colour developed in day light**
Powder as such	Green
1N NaOH	Greenish brown
Picric acid	Yellowish green
Glacial acetic acid	Yellowish green
1N Hcl	Pale yellowish green
1N HNO_3	Pale yellowish green
5%Iodine	Yellowish green
40% NaOH +few drops of 10% lead acetate	Yellowish green
HNO_3+ Ammonia solution	Yellow
Con H_2SO_4	Brown
5% $Fecl_3$	Reddish brown
10% sodium hydroxide +copper sulphate	Green
Acetic acid +Con H_2SO_4	Green
Acetic acid +Ferric chloride +Con H_2SO_4	Dark blackish brown
Antimony tri chloride	Green
Ammonia solution	Pale green

Similarly, the fluorescence characteristic of the powdered leaf, when treated with various chemical reagents (Table 3) and its extracts (Table 4), have also been extensively studied.

Table 3. Fluorescence characters of the powdered leaves of *bauhinia purpurea* under UV light .

Treatments	**Colour Developed Under UV Light**	
	Short (254nm)	**Long (366 nm)**
Powder as such	Buff	Pale green
1N HNO_3	Dark green	Pale green
5N NaOH in water	Pale green	Pale green
1N Hcl	Pale brown	Pale green
50% HNO_3		Green Green
Acetic acid	*Grey*	Pale green

Picric acid	Dark green	Pale green
$Fecl_3$ (5%w/v aqueous solution)	Black	Bluish black
N/20 Iodine	Black	Greenish black
50% H_2SO_4	Dark green	Yellowish green
Ethanol	Green	Green
1N NaOH in ethanol	Reddish yellow	Yellow
Methanol	Green	Dark green
Powder mounted with nitro cellulose	Grayish white	Greenish brown
Powder treated with NaOH in methanol, dried and mounted with nitro cellulose	Yellowish green	Dark green
Powder treated with HCl, dried and mounted with nitro cellulose	Greenish yellow	Yellowish green
Powder treated with NaOH in water and mounted with nitro cellulose	Brown	Reddish brown
Powder treated with Antimony tri chloride	Green	Brown

Table 4. Fluorescence analysis of different extracts of *bauhinia purpurea.*

	Colour Developed Under UV light	
Extract	**Long (366 nm)**	**Short (254 nm)**
Petroleum ether	Light green	Greenish yellow
Chloroform	Greenish brown	Dark greenish brown
Acetone	Dark green	Light green
Ethyl acetate	Blackish green	Black
Methanol	Yellowish green	Green
Water.	Greenish buff colour	Dark green

Fluorescence studies on extracts revealed different shades of green fluorescence under UV light at 254nm and 365nm. After successive extraction with each solvent by using the soxhlet apparatus , the percentage of dry extract was calculated in terms of air-dried weight and reported: Petroleum ether (6.68 %); Chloroform (1.72%); Acetone (3.24%); Ethyl acetate (2.69%); Methanol (17.93%) ; Water (18.58%), etc. The chloroform extract was minimum whereas the water extract showed the maximum extractive value, thus indicating the presence of more polar constituents in the leaf extract. Also noted were the colour and consistency of the extracts (Table 5).

Table 5 . The colour and consistency of leaf extracts of *bauhinia purpurea*.

S.No.	**Extracts**	**Colour**	**Consistency**
1	Petroleum ether (60 – 80^{o}c)	*Greenish black*	*Semisolid*
2	Chloroform	Greenish black	Semisolid

3	Acetone	Greenish brown	Semisolid
4	Ethyl acetate	Yellow	Semisolid
5	Methanol	Dark brown	Semisolid
6	Water	Dark brown	Sticky

To determine powder characters of 40- mesh size, the angle of repose and bulk density were also calculated. The drug showed an angle of repose of 44°27” and 0.4413 g/p.c. bulk density.

The various qualitative chemical tests (Table 6) have shown the presence of phytosterols, flavonoids, fixed oils, phenolic, tannins, glycosides and saponins in huge amount; whereas, alkaloids, aromatic acids, carbohydrate, proteins and aminoacid, triterpenoids, gums, mucilage and volatile oils were totally absent in the leaf extract of this plant.

Table 6. Preliminary phytochemical screening of various extracts of *bauhinia purpurea.*

SI.No	Plant Constituents	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Acetone extract	Methanol extract	Aqueous extract
1	Alkaloids	-	-	-	-	-	-
2	Carbohydrates	-	-	-	-	-	-
3	Glycosides	-	+	-	-	+	-
4	Saponins	-	-	-	-	+	+
5	Proteins & Amino acids	-	-	-	-	-	-
6	Phenolic compounds & Tannins	+	-	-	+	+	+
7	Gums & Mucilage	-	-	-	-	-	-
8	Flavanoids	+	+	+	+	+	+
9	Fixed Oils and Fats	+	+	+	+	+	-
10	Volatile oils	-	-	-	-	-	-
11	Triterpenoids	-	-	-	-	-	-
12	Phytosterols	+	+	+	+	+	+

13	Aromatic acids	-	-	-	-	-	-
	(+) Denotes	presence					
	(-) Denotes	absence					

Conclusion

Macroscopic as well as microscopical studies of any phytodrug are the primary steps to establish its botanical quality control before going to other studies. As per WHO norms , botanical standards are to be proposed as a protocol for the diagnosis of the herbal drug. The above mentioned parameters are helpful for the future identification and authentification of the plant in the herbal industry and in factories. The physico-chemical standards, such as ash values, extractive values, crude fiber content and fluorescence analysis, will be useful to identify the authenticity of the drug even from the crushed or powdered plant materials. It will serve as a standard data for the quality control of the preparations containing this plant in future. The leaf constants can be included as microscopical standards in Indian herbal pharmacopoeia. Phytochemical study is also useful to isolate the pharmacologically active principles present in the drug. The information obtained from the ash values and extractive values are useful during the time of collection and also during extraction process. Using these standards, the plant can be differentiated from other related species.

Acknowledgements

The authors are grateful to the Director, NISCAIR, New Delhi for assistance in literature collection of this plant. They are also thankful to Dr. P. Jayaraman, Director, plant anatomy research centre (PARC), chennai for his help in the authentification of the plant material.

References

1. *The Wealth of India: Raw materials*, **VoI.II** (Publication and Information Directorate, CSIR, New Delhi) 1988, 53-54.

2. Yadava R N and Tripathi P, A novel flavone glycoside from the stem of *Bauhinia purpurea*, *Fitoterapia*, **71** (2000), 88-90.

3. Yadav R and Sodhis N, Phytochemical studies on *Bauhinia racemosa* lam.*Bauhinia purpurea* Linn, *E J Chemistry*, ***4*** (2007),123.

4. Chopra R N, Nayar S L, and Chopra I C, *Glossary of Indian Medicinal Plants* (Publication and Information Directorate, CSIR, NewDelhi) 1992 , 35.

5. Nandkarni K M, *Indian Materia Medica,* **Vol.I** (Popular Prakashan Pvt Ltd, Bombay) 1995, 182.

6. Asima Chatterjee, Satyesh Chandra Pakrashi, *The Treatise of Indian Medicinal Plants*, **Vol.2** (Publication and Information Directorate, CSIR, New Delhi) 1992, 16-21.

7. Manoranjan Sharma H, Radhapyari Devi A, and Manihan Sharma B, Vegetable drugs used by the Meitei

community of Manipur, *Indian Journal of Traditional Knowledge*, **4** *(*2005),42.

8. Mukherjee P K, Gopal T K, Subburaju T, Dhanbal S P , Duraiswami B, Elango and Suresh B, Studies on the anti-diarrhoeal profiles of *Bauhinia purpurea* Linn Leaves(Fam. Caesalpinaceae) extract, *Natural Product Science*, **4** (1998),234-237.

9. Pettit G R, Numata A, Iwamoto C, Usami Y, Yamada T, Ohishi H and Cragg G M, Antineoplastic agents. 551. Isolation and structures of bauhiniastatins 1-4 from *Bauhinia purpurea*, *J Nat Prod* , **6** (2006), 323-7.

10. Panda S and Kar A, *Withania somnifera and Bauhinia purpurea* in the regulation of circulating thyroid hormone concentrations in female mice, *J Ethno pharmacol*, **7** (1999) ,233-9.

11. Wallis T E , *Textbook of Pharmacognosy* (CBS publishers and Distributors, Delhi) 1985, 104 – 105.

12 Khandelwal K R , Kokate C K , Pawar A P and Gokhale S R, *Practical Pharmacognosy Techniques and Experiments* (Nirali Prakashan Publishers, Pune)1996, 9.

13. Anonymous, *The Indian Pharmacopoeia* (Govt. of India publication, New Delhi) 1966, 947-950.

14. Wallis T E , *Text Book of Pharmacognosy* (CBS publishers and Distributors, Delhi) 1989, 356 – 549.

15. Brain K R and Turner T D , The practical evaluation of phytopharmaceuticals, *Wright-Scientechnia*, **6** (1975), 81.

16. Kokoshi J, Kokoski R and Slama F J , Fluorescence analysis of powered vegetable drugs under ultraviolet radiation , *J Am Pharm Assoc*, **47** (1958), 75-77.

17. Johansen D A, *Plant Microtechnique* (McGraw Hill Book Co., New York) 1940, 182-197.

18. Harborne J B , *Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis* ,(Chapman and Hall, London) 1973, 182-189.

19. Cooper J , Gunn C , Powder flow and compaction. In: Lachman I. Liberman H A, Kanig J L, *The Theory and Practice o f Industrial Pharmacy* (Vargheese Publishing House, Mumbai) 1986 430-456.

20. Sourabh J , Manish L , Nayak S, Preliminary phytochemical studies on the roots of *Cocculus hirsutus* Linn, *Ancient Science of Life***, 3** (2004),42-45.

21. Trease G E and Evan W C , *Pharamcognosy* (ELBS Publications, London) 1985, 344 and 539-540.

22. Anes Ahmad Sidiqui and Mohammed Ali , *Practical Pharmaceutical Chemistry* (CBS publishers, New Delhi) 1997, 127-137.

23. Tyler V E, Brady L R and Robber J E , *Pharmacognosy* (Lea and Febiger Publication, Philadelphia) 1988, 77-79.

24. Kokate C K , *Practical Pharmacognosy* (Vallabh Prakashan, New Delhi) 1991, 107-111.

25. Peach and Tracey M V. , *Modern Methods of Plant Analysis,* **Vol. III** (Springer and Verlag, Berlin) 1955, 321-322.