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6 Pharmacognostic Study and Phytochemical Evaluation of *Barleria cuspidata* Heyne ex Nees

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ABSTRACT

Barleria cuspidata Heyne ex Nees., (Acanthaceae) has been used to heal maceration of feet, stomach ache, tooth ache, mouth sores, teeth problems, etc., and is known for its antioxidant, antidiabetic, antihyperlipidemic, hepatoprotective, and wound healing potential in folk medicine. The present research investigated the macroscopic, organoleptic, and fluorescence evaluation of the leaf powder, standardization of solvents for better extraction and estimation of phytochemical components of B. cuspidata extracts. Fluorescence analysis showed different colors of powders when treated with different chemicals. The maximum yield was exhibited at 24h of extraction in methanol (20.53± 0.15% w/v), ethanol (20.15±0.06% w/v), aqueous (31.37±0.05% w/v), and acetone (6.74±0.04% w/v). The phytochemical investigation done showed the presence of numerous chemical compounds including alkaloids, cardiac glycosides, tannins, flavonoids, and phenols in all the extracts.

Keywords: *Macroscopic, Organoleptic, Standardization, Phytochemicals, B. cuspidata.*

INTRODUCTION

Plants have been used for medicines and health care purposes long before the prehistoric period. According to WHO, it has been estimated that around 21,000 plant species have the potential for being used as medicinal plants, and 80 percent of people worldwide depend on herbs for medicines as their primary health care needs. In India, due to its rich repository of medicinal and aromatic plants, traditional medicine is treated as a priority for healthcare. *Barleria cuspidata* Heyne ex Nees is a perennial, armed shrub that grows up to 1m long under dry plains and rocky hill slopes. The tips of leaves and bracts are spiny in nature and hence called Spiny Barleria. In Ayurveda, it is known as Bajradanti and is used for various medicinal purposes. The juice of the roots and leaves is used to heal maceration of feet, stomach ache, tooth ache, mouth sores, teeth problems, and the twig is used as a toothbrush for strengthening teeth and gums. The leaves of *B.cuspidata* are mixed with pepper and used to relieve tooth aches. Roots and leaves are used in folk medicines to cure cough and bronchitis. All the parts of the plant possess various chemical constituents like alkaloids, carbohydrates, flavonoids, flavones, glycosides, steroids, tannins, phenols, etc. (Tamilselvi, 2017). The roots and leaves were used traditionally in stomach ache, tonic, febrifuge, cough, bronchitis, and inflammation (Madhavachetty *et al*., 2013). *B.cuspidata* is proved for wound healing activity (Mazumder *et al*., 2009), hepatoprotective activity, antioxidant (Tabassum *et al*., 2020), antidiabetic and antihyperlipidemic activity (Reddy and Sundararajan 2020, 2021). These health benefits could be a part attributed to the potential effects of their antioxidants such as phenolic compounds on the reactive oxygen species produced in the human body.

Morphological description:

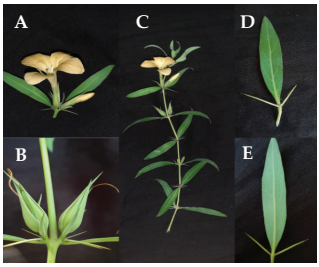

Fig.1: Parts of the plant ***B.cuspidata*****.**
A) Flower, B) Nodes and thorns, C) Twig,
D) Ventral and E) Dorsal side of the leaf.

Barleria cuspidata is a perennial, spiny branched shrub about 1m tall. Stem of this plant are branched, terete, or obscurely tetragonous, glabrous, and 4-angled. Nodes are bristle and two to four thorns are present at axillary up to 2 cm long. Petioles are 5-10 mm long having opposite leaves of size 14.5 x 2 -6 cm, which are ovate to elliptic in shape, attenuate at the base, and have acuminated apex. Venation is very distinct on both the midribs, grooved above, with 3-6 pairs of lateral nerves and are glabrescent. The flowers of this plant are solitary with terminal spikes. The length of the flower is 6-6.5 cm having a bright yellow color. Bracts are white pubescent, linear-lanceolate size 1-2.2 x 0.2-0.5 cm with a cuspidated apex. This plant possesses two whorls of sepals, of which the outer is ovate-lanceolate, sub-equal, glandular-pubescent inside, and palmately nerved. The inner sepals are linear-lanceolate, as long as the outer and 3-4 mm across with acute apex. Corolla bilipped with an upper lip of 4 obovate, acute lobes; the lower lip is shorter than the upper, obovate, acute, bent at base, hairy outside, and obtuse at apex. Two fertile stamens are present with anthers of 4mm long, and flattened hairy filaments with a length of 4.8-5.5 cm are found exerted beyond the corolla tube. Ovoid ovary of 3-4 mm long, glabrous possessing a slender style of length 5.5-6.5 cm and stigma ovate, papillate of 1 mm long. *Barleria cuspidata* have capsules ovoid in the shape of 1.5-2 x 0.6-0.8 cm. Seeds of *B. cuspidata* are two in number, orbicular, 8.5-10.8 x 6.5-8 mm, sub-cordate at base, compressed on one side, with silky appressed hairs, and are copper-brown in color (Gamble, 1924).

MATERIALS AND METHODS

Collection of plant material:

The plant *Barleria cuspidata* Heyne ex Nees was selected based on knowledge gained from the tribal people and collected between January and March 2020, during its flowering period from Thottakombai hill which is situated in Erode district of Tamil Nadu. The fresh plant specimen (Voucher No: BSI/SRC/5/23/2020/Tech/685) was given for authentication and identified by the Botanical Survey of India, Southern Regional Centre, TNAU Campus, Coimbatore. A bunch of collected leaves was washed in sterile water, dried under shade, pulverized, and stored at 4°C for further use.

Macroscopic and Organoleptic evaluation of *B. cuspidata* leaf powder:

Fresh leaf material was used for macroscopic evaluation. The macroscopic characters such as surface, shape, size, color, venation,

phyllotaxy, petiole length of *B. cuspidata* leaf were noted. The dried leaf powder was used for the organoleptic study. The sensory parameters like color, odor, and the taste of the leaf were examined.

Fluorescence analysis of *B. cuspidata*:

Fluorescence analysis of leaf powder was done by standard procedure (Edwin, 2008). The fluorescence characters of powdered drugs play an important role in the identity, safety, purity, and quality of the plant material. 1gm of plant powder was taken in a petri dish, treated with various acids, and alkali and observed in the transilluminator under visible, short wave, and longwave regions of Ultraviolet light and recorded for the color change.

Standardization of Solvent for Extraction:

The powdered samples were weighed (10g), tightly packed in Whatman No.1 filter paper, and extracted using various solvents like methanol, ethanol, acetone, and aqueous (100ml) for different periods of time (6, 12, 18 and 24 hours) using soxhlet extraction. The solvent-extracted (Methanol, Ethanol, Acetone) fractions were subjected to a rotary evaporator and the aqueous extract was subjected to lyophilization to remove the excess solvent. The dried leaf residues were weighed and stored at 4°C in an air-tight glass container for further tests. The final extraction yield was calculated using the formula,

$$\text{Extraction Yield (\% w/v)} = \frac{\text{Weight of the residue obtained}}{\text{Weight of the plant material taken}} \times 100$$

Preliminary Phytochemical Screening:

The stock solution from all the solvent extracts (100 mg) were prepared and dissolved in 10 ml of its own mother solvents and subjected to preliminary phytochemical screening (Harborne, 1998).

Qualitative estimation:

The extracts obtained were then subjected to qualitative chemical tests for identification of various plant constituents like carbohydrates, reducing sugar, sterols, resins, tannin compounds, saponin, alkaloids, flavonoids, terpenoids, glycosides, phenols, steroids, acidic compounds, catechin, oils, cardiac glycosides, protein, anthraquinone, amino acids, phlobatannin, quinone, starch, carotenoid, oxalate, and vitamin C.

RESULTS AND DISCUSSION

The result of macroscopic and organoleptic (color, odor, texture, and taste) observations of the leaf are the important identification tools for *B. cuspidata* (Table- 1).

Table 1. Macroscopic and Organoleptic study of the fresh leaf of *B.cuspidata*

Evaluation	Characters	*B.cuspidata*
Macroscopic evaluation	Size	14.5 x 2 -6 cm
	Shape	ovate to elliptic
	Base	Attenuate
	Apex	Accuminate
	Node	2-4, Bristled
	Texture	Rough
	Margin	Entire
	Venation	Reticulate
	Petiole	5-10 mm
Organoleptic evaluation	Color	Green
	Odour	Characteristic
	Texture	Glabrous
	Taste	Bitter

The plants can be identified based on their macroscopic and organoleptic characters. However, it becomes the preliminary step in the raw drug standardization techniques and also provides the authentication of the original source (Joseph and Simon, 2019).

Table 2. Fluorescence analysis of the leaf powder of *B.cuspidata.*

S. No	Treatment with chemical reagents	Visible light	UV- Short wave (254 nm)	UV- Longwave (365 nm)
1.	Crude powder	Dark green	Light green	Light green
2.	Powder with 1N HCL	Dark green	Fluorescent green	Ceramic green
3.	Powder with 1N NaOH in MeoH	Dark green	Fluorescent green	Blackish green
4.	Powder with 50% HNO_3	Dark green	Green	Dark green

5.	Powder with acetic acid	Brown	Light Brown	Reddish Brown
6.	Powder with picric acid	Yellowish green	Oily green	Fluorescent green
7.	Powder + 1 % $FeCl_3$	Dark green	Blackish green	Blackish green
8.	Powder with 1N NaOH	Green	Dark green	Blackish green
9.	Powder treated with Con. H_2SO_4	Dark green	Green	Ceramic green
10.	Powder with water	Dark green	Fluorescent green	Fluorescent green

The chemical compound present in most of the plants exhibits the characteristics of fluorescence and it can be used as a standard parameter for quality control of the drug and to detect adulterants in crude drugs (Gayathri and Kiruba, 2015) `(Kasthuri and Ramesh, 2018). The fluorescence analysis of these leaf powder was observed under visible light, UV- Short wave (254 nm), and UV- Longwave (365 nm) light. It expressed different shades of green color like dark, light, fluorescent, blackish, ceramic, yellowish and oily green and also absorbed brown, light brown and dark brown when treated with various chemical reagents. (Table - 2).

Fluorescence analysis is one of the pharmacognostic procedures which helps to identify the exact samples and recognize the adulterants (Tyler, 1976). The powder showed green fluorescence in UV light, which indicates the presence of chromophores in the drug (Arumugam and Natesan, 2015).

Table 3. Percentage of extraction yield

S. No	Solvent	Extraction Yield (%) (w/v)			
	Time (h)	Methanol	Ethanol	Acetone	Aqueous
1.	6 Hrs	17.71±0.23	15.09±0.030	4.15±0.13	15.53±0.25
2.	12 Hrs	18.16±0.12	18.16± 0.04	4.26±0.04	20.14±0.025
3.	18 Hrs	18.96± 0.15	16.92±0.06	6.13±0.03	25.68±0.03
4.	24 Hrs	20.53± 0.15	20.15±0.06	6.74±0.04	31.37±0.05

Values are the mean ± SD of triplicates

The above results which are shown in Table 3. imply that there is a certain correlation between time and extraction yield. Extraction yield gained from aqueous, methanol, and acetone solvents expressed the gradual increase in the percentage as time increases from 6h to 24h, except for the ethanol extraction, in which the outcome was not increased with the increase of time. The maximum yield was exhibited at 24h of extraction in methanol (20.53± 0.15% w/v), ethanol (20.15±0.06% w/v), aqueous (31.37±0.05% w/v), and acetone (6.74±0.04% w/v). Thus, we can conclude that the optimal extraction time depends on the solvent type.

This observation was supported by Fick's second law of diffusion, the final equilibrium will be achieved between the concentrations of the solute particles in the plant matrix and in the bulk solution (solvent) increase in the extraction time did not extract more compounds and also oxidation occurs due to light or oxygen exposure (Chan *et al.*, 2009). This trend was similar to solvent standardization of *Barleria prionitis* extract which exhibited an increased yield in aqueous extract (Leaf 8.40% and Stem 6.73%) with the increase in polarity of solvent used for the extraction (Sharma *et al.*, 2014).

Table 4. Qualitative analysis of Phytoconstituents Present in all the Extracts of *B.cuspidata* Heyne ex. Nees.

S. No	Phytocompounds	Methanol	Ethanol	Acetone	Aquoeus
1.	Alkaoids	++	++	+	+
2.	Tannins	++	++	+	++
3.	Phenols	++	+	++	++
4.	Saponins	+	++	+	+
5.	Proteins	++	++	++	++
6.	Anthocyanin	++	++	+	+
7.	Quinone	-	-	+	-
8.	Oxalate	++	+	++	-
9.	Flavanoids	++	+	+	+
10.	Vitamin C	-	-	++	++
11.	Carbohydrates	++	++	++	++
12.	Phytosterols	+	++	-	-
13.	Coumarins	++	++	++	+

14.	**Terpenoids**	++	+	+	++
15.	**Volatile oil**	+	+	+	-
16.	**Resins**	++	+	+	++
17.	**Carotenoids**	+	++	++	-
18.	**Cardiac Glycosides**	+	++	++	+
19.	**Phlobatanin**	-	-	-	++
20.	**Catechin**	+	+	+	+
21.	**Reducing sugar**	+	++	+	+
22.	**Amino acids**	++	++	+	++
23.	**Starch**	+	+	+	-
24.	**Acidic compounds**	++	++	++	-

+(present), ++(Highly present), -(Absent)

The presence and absence of various phytochemicals screened using methanol and aqueous plant extracts were depicted in Table 4. Results were interpreted with a visual appearance like change in color, precipitate, and ring formation in the addition of certain chemicals to the plant extracts. Methanol and ethanol extract showed the presence of most of the phytocompounds except quinone, vitamin C, and phlobatanin. The twenty two tested compounds were found to be present in acetone extract, while it did not contain phytosterols and phlobatanin. Aqueous extract tested negative for the presence of quinone, oxalate, phytosterol, volatile oil, carotenoids, starch, and acidic compounds and revealed a positive test for all the other phytocompounds.

Flavonoid, sterol, alkaloid, and phenolic compounds are found in methanol extract and alkaloids, reducing sugars and phenolic compounds are present in aqueous extracts of *B. lupulina* (Mazumder, *et al.,* 2014). In contrast, the least chemical constituents like alkaloids, carbohydrates, glycosides, steroids, flavonoids, and phenolic compounds are noticed n-hexane, ethyl acetate, and methanol leaf and stem extracts of *B. prionitis* (Sharma *et al.,* 2014). Similar results have been recorded from methanol leaf extracts of *B. lupulina* (Kumari *et al.,* 2017). Tamilselvi *et al.,* recorded the presence of alkaloids, terpenoids, triterpenoids, esters, aliphatic ketones, β-carotene, and so on in the species *B.buxifolia*.

CONCLUSION

Quality analysis of crude drugs and standardizing them for extraction plays a principal role in evaluating their medicinal properties. Aqueous and methanol were analyzed to be high extracting solvents and phytochemical screening showed the presence of most of the tested compounds except a few in all the extracts. Thus, this study further ensures that the plant has potential chemical compounds which need to be analyzed to enhance its therapeutic properties.

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