

Pharmacognostic and phytochemicals evaluation of *Blumea* species

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Abstract

Blumea malcolmii Hook f. Family (Asteraceae) commonly known as “Panjrut” in Marathi is a small herb stem covered with rough wool. It is folklore medicine generally used for wound healing activity. The present study reveals to investigate various parameters such as qualitative analysis on leaves of *Blumea malcolmii* Hook f. The qualitative studies involves microscopic and phytochemical analysis. Phytochemical characterization of chloroform, ethanol, methanol and water extract of leaves revealed presence of Saponins Glycoside, Volatile oil, flavonoids glycoside, Alkaloid and Tannins. The result of the study can served as a valuable resource of Pharmacognostic and phytochemical information.

Keywords: pharmacognostic, phytochemical, evaluation, *Blumea* species

Introduction

Genus *Blumea* is found in the topical and sub-topical zones of Asia, especially the Indian sub-continent and Southeast Asia. A few species are found in Australia and still fewer in Africa. The plants of this genus are mostly relatively small weed. Some of them are ruderal species. Many species of genus *Blumea* are used in traditional Chinese medicine.

Blumea malcolmii Hook F, Family - Astraceae is a small herb, stem covered with rough wool. It occurs commonly on Hills slopes and in open exposed area grasses of the Western Ghats. The leaves are Sessile, Obovate–Oblanceolate, Apex obtuse, margin irregularly toothed to 12 x 6 cm. Basal leaves are larger than the upper leaves and has pleasant Camphoraceous smell. In folk medicine, the plant is used for the treatment of wound healing, cough, bronchitis, dysentery.

Objective

- To evaluate pharmacognostic and phytochemical characteristic of leaves of *Blumea malcolmii* Hook f
- Qualitative estimation of leaves of *Blumea malcolmii* Hook f

Literature Review

Malcom's *Blumea* is a stout herb, 1-2 ft. tall, strongly aromatic, with tap roots. Stems are much branched from base, erect or rising up, densely woolly with silky white hairs. Leaves are obovate to invert –landscaped, 1-12 cm x 0.7-5 cm, closely irregularly spiny–saw-toothed, blunt, densely white woolly on both surfaces, stalk less. Flower–heads are borne in branch–end fascicles, clustered at the ends of branches, 7-11 mm across. Flower –cluster–stalks are up to 2 cm long, densely woolly; involucre bracts linear, 2-8 mm long, pointed, reflexed at maturity, densely wholly on dorsal surface; receptacle convex, alveolate, hairless; central florets bisexual, with tubular flower, 6.5–7 mm long, 5-lobed, yellow, velvet hairy; marginal florets female, thread like, 5.5–6 mm long, 3 lobed, velvet–hairy. Achenes are brown, ribbed, silky, shining, velvet–hairy, 1.5-1.8 x

0.5 - 0.6 mm. Pappus white, 6 - 6.7 mm long, berbellate. Flowering: November - February.

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Material and Method

Plant material

The plant specimens (fresh leaves) of *Blumea malcolmii* were collected from campus of Yadavrao Tasgaonkar Group Of Institute, Bhivpuri road during November 2019. The collected plant were carefully examined and authenticated by

Preparation of *Blumea malcolmii* Hook f extract

The collected leaves were washed with running tap water to remove adhering material and cut into small pieces. Then, the leaves were dried at temperature not exceeding 45°C. These dried material were cut into small pieces and the pulverized mechanically into powder. The fine powder obtained by passing through sieve no 60. Then this powder obtained by passed through the sieve no. 18. Then this fine powder was extracted by macerated with different solvents like ethanol, methanol, chloroform and water. The different extract were concentrated with electric water bath at temperature not exceeding 45°C and stored in Vials. ^[1]

Qualitative Estimation

Microscopic analysis

Powder microscopy is one of the simplest and cheapest methods to start with for establishing the correct identity of the *Blumea malcolmii* Hook f.^[2]

Phytochemical analysis

Basic phytochemical screening was carried out using simple chemical test to detect the presence of secondary plant constituents such as alkaloids, tannins, flavonoids, saponins, and glycoside etc. The methods used were those outlined by Practical Pharmacognosy by Dr. KR Khandelwal.

Tests for carbohydrates

- a. **Molisch's test (General test):** To 2-3 ml aqueous extract, add few drops of alpha-naphthol solution in alcohol shake and add conc. H_2SO_4 from sides of the test tube. Violet ring is formed at the junction of two liquids.
- b. **Fehling's test (Tests for reducing sugar):** Mix 1 ml Fehling's A and 1ml Fehling's B solutions, boil for one minute. Add equal volume of test solution. Heat in boiling water bath for 5-10 min. First yellow, then brick red ppt. is observed.

Tests for proteins

- a. **Biuret test (General test):** To the 3ml T.S add 4% NaOH and few drops of 1% $CuSO_4$ solution. Violet or pink colour appears.
- b. **Millon's test (for protein):** Mix. 3ml T.S with 5ml of Million reagents. White ppt. warm ppt. turns brick red or the ppt. dissolves giving red coloured solution.

Tests for steroid

- a. **Salkowski reaction:** To 2ml of extract, add 2ml chloroform and 2ml conc. H_2SO_4 . Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.
- b. **Liebermann-Burchard reaction:** Mix 2ml extract with chloroform. Add 1-2ml acetic anhydride and 2 drops conc. H_2SO_4 from the side of test tube. First red, then blue and finally green colour appears.

Tests for anthraquinone glycosides

- a. **Borntrager's test (for o-glycoside):** To 3ml extract, add dil. H_2SO_4 . Boil and filter. To cold filtrate add equal volume benzene or chloroform. Shake well, separate the organic solvent. Add ammonia. Ammoniacal layer turns pink or red.
- b. **Modified Borntrager's test (for c-glycoside):** To 5ml extract, add 5ml 5% $FeCl_3$ and 5 ml dil. HCl. Heat for 5 min in boiling water bath. Cool and add benzene or any organic solvent. Shake well. Separate organic layer, add equal volume dilute ammonia. Ammoniacal layer show pinkish red colour.

Test for Saponins glycoside

- a. **Foam test:** Shake the drug extract or dry powder vigorously with water. Persistent stable foam is observed.

Tests for flavonoids

- a. **Shinoda test:** To dry powder or extract, add 5 ml 95% ethanol/t-butyl alcohol, few drops conc. HCl and 0.5 g

magnesium turning. Oranges, pink, red to purple colour appears (flavonoids, dihydro derivatives and xanthenes).

- b. **Alkaline test:** Addition of increase amount of sodium hydroxide to the residue shows colouration which decolourises after addition of acid.

Tests for tannins and phenolic compounds

To 2-3 ml of aqueous or alcoholic extract, add few drops of following reagents:

- a. **5% $FeCl_3$ solution:** deep blue black colour.
- b. **Lead acetate solution:** white ppt.

Tests for alkaloids

Evaporate the aqueous, alcoholic and chloroform extract separately. To residue, add dilute HCl. Shake well and filter. With filtrate, perform following tests:

- a. **Dragendorff's test:** To 2-3ml filtrate, add few drops of Dragendorff's reagent. Orange brown ppt. is formed.
- b. **Mayer's test:** To 2-3ml filtrate, add few drops of Mayer's reagent gives ppt.^[3]

Other Parameter Estimation

Botanical parameters

Sensory evaluation

It includes visual macroscopy and touch, odor, taste etc.^[9]

Foreign matter

Procedure

1. Weigh 100 to 500 g of the sample.
2. Spread the sample on a white till or a glass plate uniformly without overlapping.
3. Inspect the sample with naked eyes or by means of a lens (5x or above).
4. Separate the foreign organic matter manually.
5. After complete separation, weigh the matter and determined % w/w present in the sample.^[9]

Physico-chemical parameters

Ash value

Determination of total ash value

Procedure

1. Weigh and ignite flat, thin, porcelain dish or a tared silica crucible.
2. Weigh about 2gm of the powdered drug into the dish/crucible.
3. Support the on a pipe-clay triangle placed on a ring of retort stand.
4. Heat with a burner, using a flame about 2cm high and supporting the dish about 7cm above the flame, heat till vapours almost cease to be evolved; then lower the dish and heat more strongly until all the carbon is burnt off.
5. Cool in a desiccator.
6. Weigh the ash and calculate the percentage of total ash with reference to the air dried sample of the crude drug.

If a carbon free ash cannot be obtained in this way then any one of the following method can be used.

1. Exhaust the charred mass with hot water, collect the residue on an ash less filter paper incinerate the residue and filter paper, add the filtrate, evaporate to dryness and ignite at a temperature not exceeding 450°C.

- Cool the crucible; add 15ml of alcohol break up the ash with glass-rod burn off the alcohol and again heat the whole to a dull red heat. Cool, weight the ash.

Calculation

Weight of the empty dish = x

Weight of the drug taken = y

Weight of the dish + ash (after complete incineration) = z

Weight of the ash = (z-x) g

'y' g of the crude gives (z-x) g of the ash

100g of the crude drugs gives $\frac{100}{y} \times (z-x)$ g of the ash

$$\text{Total ash value of the sample} = \frac{100(z-x)}{y} \%$$

Determination of acid-insoluble ash value

Procedure

Proceed as per the steps mentioned in the procedure for determination of total ash value of a crude drug. Further-

- Using 25 ml of dilute hydrochloric acid wash the ash from the dish for total ash into 100 ml beaker.
- Place mere gauze over a Bunsen burner and boil for five minute.
- Filter through an ash less filter paper; wash the residue twice with hot water.
- Ignite a crucible in the flame, cool and weigh.
- Put the filter - paper and residue together in the crucible, heat gently until vapours ceases to be evolved and then more strongly until all carbon been removed.
- Cool in a desiccator.
- Weigh the residue and calculate the acid-insoluble ash of the crude drug with reference to the air dried sample of the crude drug.

Calculation

Similar to previous experiment.

Weight of the residue (step vi) = 'a' g

(Acid insoluble ash)

'y' of the air dried drug gives 'a' g of acid-insoluble ash.

100g of the air dried drug gives $\frac{100 \times a}{y}$ g of acid - insoluble ash.

$$\text{Acid - insoluble ash value of the sample} = \frac{100 \times a}{Y} \%$$

Determination of water soluble ash

This is determined in a similar way to acid insoluble ash, using 25ml of water, in place of dilute hydrochloric acid. [9]

Extractive value

Determination of alcohol-soluble extractives

Procedure

- Weigh about 4g of the coarsely powdered drug in the weighing bottle and transfer into a dry 250ml conical flask.
- Fill a 100ml graduate flask to the delivery mark with the solvent (90% alcohol). Wash out the weighing bottle and pour the washings, together with the remainder of the solvent into the conical flask.
- Cork the flask and set aside for 24 hours, shaking frequently. (maceration)
- Filter into 50ml cylinder. When sufficient filtrate has collected, transfer 25ml of the filtrate to a weighed, thin porcelain dish, as used for the ash value determination.

- Evaporate to dryness on a water bath and complete the drying in an oven at 105°C for 6hrs.
- Cool in desiccator for 30 minutes and weigh immediately.
- Calculate the percentage w/w of extractive with reference to the air dried drug.

Calculation

25ml of alcoholic extract gives = x g of residue

100ml of alcoholic extract gives = 4x g of residue

Since 5g of air dried gives 4x g of alcohol (90%) soluble residue.

100g of air dried gives 80g of the alcohol (90%) solute residue.

Alcohol (90%) soluble extractive value of the sample = 80x %

Determination of water-soluble extractive

Procedure

- Step (i) and (ii) are same as mentioned in the above experiment.
- Cork the flask and shake it well and allow to stand for one hour.
- Attach a reflux condenser to the flask and boil gently for one hour.
- Cool and weigh.
- Readjust to the original total weight with the solvent (water / alcohol / any other solvent) used for extraction.
- Shake well and filter rapidly through a dry filter [9].

Moisture content

Determination of loss on drying

Procedure

- Weigh about 1.5g of the powdered drug into a weighted flat and thin porcelain dish.
- Dry in the oven at 100°C or 105°C, until two consecutive weighings do not differ by more than 0.5mg.
- Cool in a desiccator and weigh. The loss in weight is usually recorded as moisture [9].

Pharmacological parameters

Foaming index

Procedure

- Take 1g of coarse powder of the plant material in 500ml conical flask.
- Add 100ml of boiling water and maintain moderate boiling for the 30 minutes.
- Cool and filter.
- Collect the filtrate/decoction in a 100ml volumetric flask and adjust the volume to 100ml by adding sufficient water.
- Pour the decoction into 10 stoppered test tube as 1ml, 2ml, 3ml, etc. up to 10ml.
- Adjust the volume of liquid in each test tube to 10ml by adding sufficient quantity of water and stopper the tubes.
- Shake test tubes in a lengthwise motion for 15 seconds (two shake per second).
- Allow test tube to stand for 15 minutes and measure the height of the foam.

Calculation

$$\text{Foaming index} = \frac{10000}{a}$$

a = volume in ml of the decoction in the test tube showing 1cm foam height ^[9].

Result and Discussion

Qualitative estimation

Microscopic analysis

1. Tomata - anomocytic type.

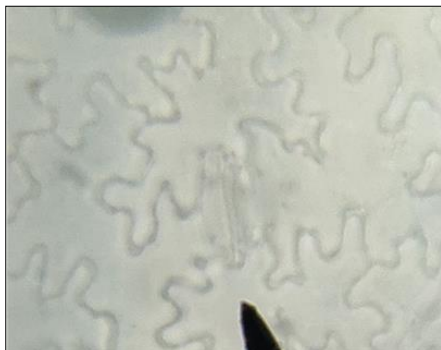


Fig 1: Anomocytic stomata

2. Trichomes

a. Uniseriate, multicellular covering trichomes

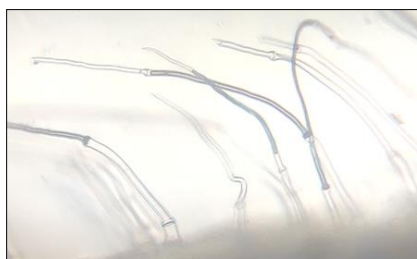


Fig 2: Covering Trichomes

b. Glandular Trichomes.



Fig 3: Glandular Trichomes

3. Xylem-annular spiral.



Fig 4: Xylem vessel

4. Oil Cells



Fig 5: Oil cells

5. Calcium oxalate crystal-prismatic crystal



Fig 6: Calcium oxalate crystal

Phytochemical analysis

Table 1: Phytochemical analysis

Sr.no	Test	Methanol extract	Ethanol extract	Choloroform extract	Water extract
1.	Carbohydrate-molisch test	-	-	-	-
	▪ Fehling test	-	-	-	+
2.	Protein-biuret test	-	-	-	-
	▪ Millon test	-	-	-	-
3.	Steroid-salkowski test	+	-	+	-
	▪ Liebermann burchard test	+	+	-	-
4.	Anthraquinone glucoside-borntrager test	-	-	-	-
	▪ Modified borntrager test	-	-	-	-
5.	Saponins glycoside-foam test	-	-	+	-
6.	Flavonoids-shinoda test	+	-	-	+
	▪ Alkaline test	+	-	-	+
7.	Tannins-5% FECL3 solution	+	+	+	+
	▪ Lead acetate solution	+	+	+	+
8.	Alkaloids-dragendroff test	+	+	-	-
	▪ Mayers test	+	+	-	-

Preliminary Phytochemical screening of successive extracts indicated the presence of saponins glycoside, volatile oil, flavonoids glycoside, alkaloid and tannins

Other parameter estimation

Botanical parameter

Sensory evaluation

1. **Leaves:** sessile, obovate - oblanceolate
2. **Apex:** obtuse
3. **Margin:** irregular toothed to 12 x 6 cm
4. **Smell:** pleasant camphoraceous smell

Foreign matter

Total foreign organic matter present in *Blumea malcolmii* Hook f is about 0.28% w/w

Physico - chemical parameter

Ash value

Table 2: Total Ash value

Sr.no	Parameter	Values
1.	Total ash value	20% w/w
2.	Acid Insoluble Ash	2.1%
3.	Water soluble Ash	49%

Extractive value

Table 3: Extractive value

Sr. no	Parameter	Value
	Extractive value	
1.	Alcohol soluble extractive value	1% w/w
2.	Water soluble extractive value	59% w/w

Moisture content

Moisture content of the *Blumea malcolmii* Hook f is 5% w/w.

Pharmacological parameter

Foaming index

Foaming index of *Blumea malcolmii* Hook f is 2000

Conclusion

The results from this study have provided information on the morphological, physicochemical and pharmacological parameters of the leaves of *Blumea malcolmii* Hook f. These parameters can be used for identification and quality control of the plant.

The Pharmacognostic and Phytochemical study on the Leaves of *Blumea malcolmii* Hook.f has shown that they can be used efficiently as therapeutic agents due to its various phytochemical constituents.

Further studies are required to investigate various biological activities of the Phytoconstitunets of these plants.

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