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Pharmaco-chemical characterization of leaves of *Blumea mollis* (D. Don) merr. from Western Ghats of wayanad region of Kerala, India

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Abstract
Blumea mollis (D. Don) Merr. is one of the plant belonging to Asteraceae family traditionally used for the treatment of various ailments. The present study deals with the pharmaco-chemical characterization of *Blumea mollis* (D. Don) Merr, found in Western Ghats of Wayanad region of Kerala. The physico-chemical studies showed that the plant leaves contained foreign content 0.313 %, moisture content 11.333 %, total ash content 11.416 %, acid soluble ash 56.833 %, water soluble ash 15.054 % and alcohol soluble ash 10.595%. The crude ethanolic extract and its four fractions showed the presence of biologically active phyto-constituents. The study also included preparation of different extracts by successive solvent extraction for detailed analysis. Florescence profiling of the plant powder was done under visible, short and long UV light, which signified its characteristics. The findings of the present study will be useful in setting the quality control parameters for the plant.

Keywords: *Blumea mollis*, physico-chemical, phytochemical and florescence profiling

Introduction
Blumea mollis (D. Don) Merr. is a plant belonging to Asteraceae family. It is an annual aromatic herb, with erect stems and soft glandular hairs. The leaves are ovate- oblong, petiolate and irregularly toothed. The flowers are pink purple in colour, bracts are narrowing, acuminate and achenes are terete. They grow up to 0.3-1.0 m high and are commonly found in the tropical south India, Myanmar, China, South of Sahara, Malaysia and South America. Many members of the Asteraceae family are of economic or medicinal value because of the presence of ethereal and fatty oils, resins and bitter principles. In India, traditionally the leaves of the plant are used for the treatment of various ailments as skin diseases, diarrhoea [1], asthma, dropsy [2], wounds [3] and against external parasite [4]. The leaves of the plant are also reported to have antioxidant, anticancer [5], antibacterial, larvicidal [6], hepatoprotective, and anti-inflammatory properties [7]. Phytochemically, the essential oil of *B. mollis* is reported to consist of 39 constituents, the major compounds being linalool, gamma-elemene, copaene, estragole, allo-ocimene, gamma-terpinene and allo-aromadendrene [8]. In addition, alkanes n-triacontane, n-hentriacontane, 2, 3-dimethoxy p-cymene, chrysanthanone, 2, 4, 5-trimethoxyallylbenzene, methyl-5-isopropyl-1, 2-methycyclopentane carboxylate and caryophyllene oxide are also identified from the essential oil of *B. mollis* [9, 10].
However, the literature surveys have revealed that there are no reports on the pharmacognostical studies conducted on this plant. Therefore, the present study was aimed at evaluating the phytochemical profile, fluorescence and physico-chemical characterisation of the leaves of *B. mollis*.

Systematic classification of the plant
Kingdom - Plantae
Phylum - Magnoliophyta
Class - Magnoliopsida
Order - Asterales
Family - Asteraceae
Genus - Blumea
Species - *Blumea mollis*

Fig 1: Roots, stem leaves and inflorescence of *Blumea mollis* (D. Don) Merr.

Materials and Methods

Collection and identification of plant materials

The plant *Blumea mollis* (D. Don) Merr. (Figure 1) was collected from Kerala Veterinary and Animal Sciences University campus, Pookode, Wayanad, Kerala, India. It was identified and authenticated by a botanist and a voucher specimen (Accession No: CALI 6637) was deposited at Department of Botany, University of Calicut.

Physiochemical analysis

The physicochemical parameters such as the percentage of loss on drying (LOD), total ash, foreign content, moisture content, acid soluble ash, water soluble ash and alcohol soluble ash were determined as per the Indian Pharmacopoeia [10].

Preparation of crude ethanolic extract

The plant leaves were cleaned and dried at room temperature for two weeks. The dried leaves were powdered in a plant sample grinder at controlled temperature and subjected to ethanolic extraction in a soxhlet extraction apparatus attached with a rotary vacuum evaporator (M/s Buchi, Switzerland) under reduced pressure. Solvents were evaporated off by rotary vacuum evaporator at 175 mbar at a temperature in the range of 40 °C to 50 °C.

Determination of extractive values

Coarsely chopped leaves of *B. mollis* was subjected to maceration for 24 hrs in a closed flask using 100 ml of different solvent *viz.*, ethanol, chloroform, ethyl acetate, hexane, petroleum ether and distilled water. The flask was frequently shaken during the first 6 hrs and allowed to stand for 18 hrs. After 24 hrs, the contents in the flask were filtered using Whatman No: 42 filter paper. In a flat bottom shallow dish, 25ml of filtrate was evaporated to dryness, dried at 105 ^{o}C and weighed. Extractive yield percentage was calculated with reference to the air dried powder [11].

Fractionation of ethanolic extract

The crude ethanolic extract was fractionated using solvents of ascending polarity such as hexane, chloroform, n-butanol and water. The crude ethanolic extract (100g) was transferred to a separating funnel and extracted with hexane to obtain the hexane soluble fraction. The remaining hexane insoluble fraction was successively extracted with chloroform to yield chloroform soluble fraction. Further, the chloroform insoluble fraction was extracted with n-butanol and subsequently with water to yield n-butanol and aqueous fractions respectively [11]. Solvents were removed using rotary vacuum evaporator, dried at room temperature. The extracts were freeze dried (M/s Operon Co. Ltd., Korea) and stored at -20^{O}C until further use.

Analysis of primary and secondary metabolites

The crude ethanolic extract of *B. mollis* and its four fractions were tested qualitatively for the presence of different primary and secondary metabolites like steroids, carbohydrates, tannins, flavonoids, glycosides, phenolic compounds, terpenoids, saponins and alkaloids using standard methods [11].

Fluorescence analysis

The fluorescence profiling is the one of the pharmacognostic procedures useful in the identification of authentic samples and recognising impurities. It was done by placing a small quantity of dried and finely powdered leaves on a grease-free microscope slide and observing the colour changes under

visible and UV lights after treating with several drops of different freshly prepared specified reagents. The development of colour was recorded within 1-2 minutes in order to avoid drying and resultant colour change [12].

Preliminary phytochemical screening

The crude ethanolic leaf extract of *B. mollis* and its four fractions (hexane, chloroform, n-butanol and water) were partitioned to a mixture of compounds based on the polarity of each chemical constituent. The crude extract and its fractions were subjected to preliminary phytochemical analysis [11]. The phytochemical screening helps to find out the presence of important biologically active components.

Results and Discussion

Phytochemical analysis

The results of physicochemical contents of the leaves of *B. mollis* are presented in Table 1. It yielded a total ash content of 12.8 ± 0.0003 %. The foreign content was 0.083 ± 0.021 % and moisture content 8.533 ± 0.015%. Besides the percentages of acid, water and alcohol soluble ash were 24.547 ± 0.158 %, 32.285 ± 0.391% and 14.332 ± 0.560% respectively. Since ash obtained may be derived from the plant itself (physiological ash) as well as from extraneous matter, especially sand and soil adhering to the surface of the drug (non-physiological ash), it represents the inorganic part or earthy matter along with the plant. It also gives an idea about the quality and purity of the drug.

Table 1: Physiochemical characterization of the leaves of *B. mollis* (D. Don) Merr.

No.	Parameters	Values* expressed as %
1	Foreign content	0.083 ± 0.021
2	Moisture content	8..553 ± 0.015
3	Total ash	12.8 ± 0.0003
5	Acid soluble ash	24.547 ± 0.158
7	Alcohol soluble ash	14.332 ± 0.560
8	Water soluble ash	32.285 ± 0.391

*Mean of 5 readings ± SEM

Extractive yield of the plant

Extractive yields of the powdered leaves determined using different solvents such as water, ethanol, petroleum ether, chloroform, ethyl acetate and hexane are presented in Figure 2. The maximum yield was attained following extraction with ethanol followed by water, chloroform, ethyl acetate, petroleum ether and hexane in descending order. It is observed that the extractive yield from the leaves is dependent on the polarity of the solvents.

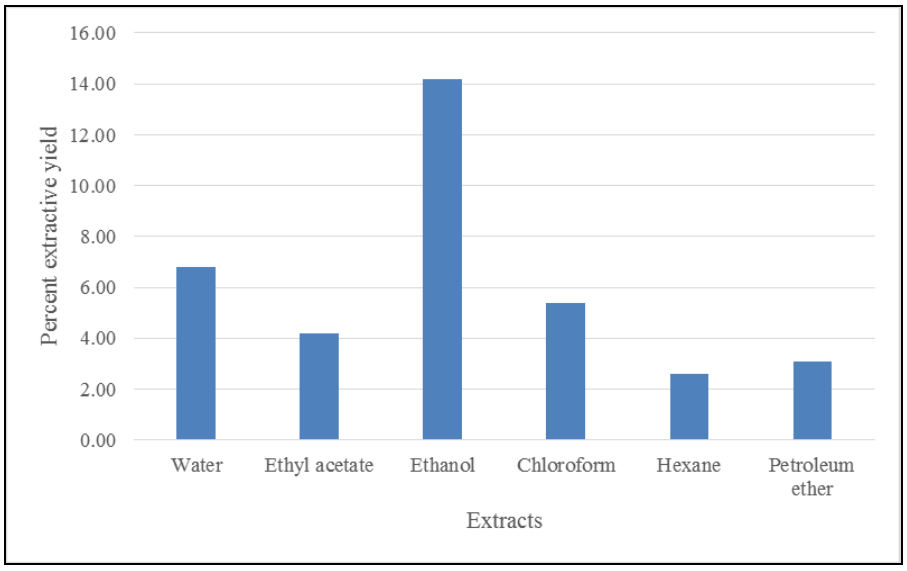

Fig 2: Extractive yield of leaves of *Blumea mollis* (D. Don) Merr. Using various solvents

Fluorescent profiling

The fluorescence profiling of the plant leaf powder was performed under ordinary light and ultraviolet light after treating with different specific chemical reagents. The powdered crude drug as such appeared greenish brown under visible light. However, it depicted black and light green under long (356 nm) and short (254 nm) UV radiations respectively. After treating with various reagents under visible and UV light, it showed different pattern of shades (Table 2).

Table 2: Fluorescence profiling of leaf powder of *B. mollis* (D. Don) Merr

No.	Reagents	Visible light	Long UV (356 nm)	Short UV (254 nm)
1	Powder	Greenish brown	Black	Light green
2	Powder + Water	Dark brown	Bluish black	Dark green
3	Powder + CH_3COOH	Brown	Greenish blue	Dark green
4	Powder + $CuSO_4$	Dark green	Bluish green	Dark green
5	Powder + HCl	Brown	Black	Dark green
6	Powder + HNO_3	Brown	Black	Dark green
7	Powder + H_2SO_4	Brown	Black	Dark green
8	Powder + $FeCl_3$	Yellowish green	Black	Greenish yellow
9	Powder + NaOH	Reddish brown	Black	Brownish green

Preliminary phytochemical screening

The crude ethanolic leaf extract of *B. mollis* and its four fractions *viz,* hexane, chloroform, n-butanol and water were subjected to preliminary phytochemical analysis. The phytochemical screening showed the presence of important biologically active components (Table 3). The crude ethanolic leaf extract of *B. mollis* qualitatively detected for the presence of alkaloids, carbohydrates, saponins, fixed oils and fat. However, it did not reveal the presence of glycosides, proteins, amino acids, tannins, phenolic compounds, gums and mucilages. All the fractions of crude ethanolic extract revealed the presence of saponins and carbohydrate. It was further noticed that the alkaloids were tested positive in the all fractions expect n-butanol fraction. The hexane and chloroform fractions further tested positive for fixed oils and fat. The phenolic compounds and tannins were only detected in hexane and water fractions. The other phytochemicals like glycosides, protein, amino acids, gum and mucilages were not qualitatively detected in any of the four fraction of ethanolic extract of *B. mollis*.

Table 3: Preliminary phytochemical screening of ethanolic leaf extract of *B. mollis* and its fractions.

No	Phytochemicals	Ethanolic extract	Different fractions of ethanolic extract			
			Hexane	Chloroform	Butanol	Water
1	Alkaloids	+ve	+ve	+ve	-ve	+ve
2	Carbohydrates	+ve	+ve	+ve	+ve	+ve
3	Glycosides	-ve	-ve	-ve	-ve	-ve
4	Saponins	+ve	+ve	+ve	+ve	+ve
5	Protein and amino acids	-ve	-ve	-ve	-ve	-ve
6	Fixed oils and fats	+ve	+ve	+ve	-ve	-ve
7	Phenolic compounds & Tannins	-ve	+ve	-ve	-ve	+ve
8	Gum and Mucillages	-ve	-ve	-ve	-ve	-ve

The presence of phenolic compounds and tannins might attribute to the larvicidal, anti-inflammatory, cytotoxicity and antioxidant properties of the plant. Phyto-constituents have already been reported with three different solvents in the leaves of *B. lacera* [12]. The aqueous extract of *B. lacera* revealed the presence of carbohydrates, amino acids, reducing sugar, tannins, phenolic compound, saponins, coumarines, and steroids. Methanolic extract revealed the presence of carbohydrates, amino acids, reducing sugar, tannins, phenolic compound, saponins, coumarines, phytosterols, terpenoids, flavonoids, and the chloroform extract indicated the presence of phytosterols, steroids, reducing sugar, flavonoids, proteins, terpenoids and alkaloids. In addition, other species of *Blumea* are also reported to contain glycosides, tannins, flavonoids, terpenoids, saponins, phenolic compounds and alkaloids [13-16]. The phytochemical screening is important and useful for isolation of pharmacologically active principles.

Conclusion

The present study reports for the first time the pharmaco-chemical characterisation of the leaves of *Blumea mollis* (D. Don) Merr. The pharmacognostic parameters reported in this study will further help in the identification, standardization of crude drug, solvent extracts and its fractions.

Conflict of interest

The authors declare that there are no conflicts of interest regarding publication of this research paper.

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