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Analgesic activity of ethanolic extract and fluorescence analysis of *Blumea mollis* (D.Don) Merr.

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ABSTRACT

Background: *Blumea mollis* belonging to the family Asteraceae and the plant has been widely used in traditional medicinal system for the cure of various diseases. It is used by people for the treatment of fever, inflammation and infections. This plant has antioxidant, analgesic cytotoxic and antibacterial property.

Materials and Methods: Plant powder extracted with different solvent *i.e.* Pet. Ether, Ethyl acetate, chloroform, ethanolic, and aqueous as per increasing polarity and extracts of the plant were prepared and analysed. The fluorescence analysis of powdered material was subjected to analysis under long ultra violet light after treatment with various chemical and organic reagents. Analgesic activity will be performed according to the Hot plate model and Tail flick model.

Results: The plant powder showed different fluorescence effects in short and long UV light and noted. The present study aimed to explore the analgesic activity of dried ethanolic extract of *Blumea mollis* (BM) using tail flick, hot plate method. Results demonstrated that BMEE exhibited a potent dose dependent analgesic activity in all tested models for analgesia

Conclusion: The results of the present study revealed that the fluorescence analysis of *Blumea mollis* can be helpful for the standardization and quality control of indigenous drug and plant good source of secondary metabolite. The BMEE extract was found to reduce pain and BMEE extract of *Blumea mollis* was found to have good analgesic activity.

Key words: fluorescence analysis, *Blumea mollis*, Analgesic activity

INTRODUCTION

Pain is a defensive reaction against abnormal of an organ or imbalance in its functions against potentially hazardous stimulus. The ascending pathway of pain in the brain that determines the locations, intensity and depth of pain. Many synthetic drugs used to relieve the pain and a few drugs like morphine and aspirin widely used but they produced pronounced side-effects on the physiology of the body. In the traditional system of medicine, several plants possess an analgesic effect (1).

Blumea mollis, family Astraceae is a genus of flowering plants distributed in Western and Southern plains of India at 2000 ft in the Himalayas [2]. *Blumea mollis* is an pleasantly fragrant annual herb 30- to 60-cm height. Its Leaves obovate, 3.5-9.5 X 1-3.5 cm, base reduce, irregular serrate margin, acute apex, pubescent on both sides. Heads in axillary, terminal corymbs. Achenes angled and sparsely hairy [3]. The leaf of the herb is traditionally used for skin diseases, and the diarrhea can be treated by boiled leaves [4]. The studies of GC-mass spectroscopy (GC-MS) showed that the leaf contained volatile oil of *B.mollis* approx 39 active constituents, and the main chemical substances were linalool, estragole, γelemene, copaene, Allo-ocimene, γ-terpinene and Alloaromadendrene [5]. The genus Blumea has reported various pharmacological effect including Anti-bacterial , anticancer, anti-oxidant, hepatoprotective, anti-microbial, anti-inflammation, anti-obesity anti-plasmodial, anti-tyrosinase, platelet aggregation, wound healing [6,7].

In light of above, in the present study, we aimed to explore the beneficial effects of *Blumea mollis* as analgesics in different analgesic animal models. The present study was aimed at evaluating the fluorescence analysis and analgesic activity of *B. mollis*.

MATERIAL AND METHOD

PLANT MATERIAL

The plant material was collected from Amauli, Fatehpur, Uttar Pradesh, India in September, 2017. The plant was identified and authenticated by the Dr. Ashok Kumar, Botanist, IFTM University, Moradabad, U.P. The plant material dried in shade were powdered and subjected to Soxhlet extraction with Pet. Ether, chloroform, Ethyl acetate, ethanol and aqueous. The extract collected was evaporated and stored in a vacuum desiccator. [14,15]

Fig. 1 *Blumea mollis* (D. Don) Merr.

DRUGS AND CHEMICALS

The following drugs Aspirin and indomethacin and chemicals, ethanol (Merck) and acetic acid (Fisher Scientific) were used during the experimental study.

QUALITATIVE ANALYSIS

Fluorescence analysis of the drug was observed under day and UV light using various solvent extracts as well as acids and alkaline treated with solutions of the drug. The powder was treated with neutral solvents like hexane, benzene, chloroform, methanol, ethyl acetate, alcohol, acetone and acids like 1N Hydrochloric acid, 50% Sulphuric acid and alkaline solutions like aqueous and alcoholic 1N NaOH [8,10].

PHARMACOLOGICAL STUDIES

ANALGESIC ACTIVITY

ANIMALS

30 Wistar Albino rats of either sex (160-200 g), six month age were used for the experimental study. The animals were maintained under standard husbandry conditions in polypropylene cages and provided with food and water *ad libitum*. The animals were kept on fasting overnight prior to the experimentation and all the procedures used in these studies were approved by the Institutional Animal Ethics Committee.

ANALGESIC ACTIVITY

Analgesic assay will be performed according to the writhing model induced by acetic acid and reported previously [9] Wistar albino rats will be used for analgesic activity. Aspirin and indomethacin will be used as positive control. Briefly , rats will be required adaptive feeding for at least a week 21± 1ºC with a 12 hrs dark/light cycle, and fasting for 12 hrs before the experiments. Then , rats will be divided into group randomly, six rats per group. One hrs after intragastric administration of test samples and the vehicle to the respective groups, rats will be intraperitoneally, injected 0.6% acetic acid solution (10ml/kg). The number of writhes of each rats in a transparent observation box will be recorded during 15 min after the acetic acid administration and percent inhibition will calculated as follows: Inhibition(100%)= [Number of writhes(control)-Number of writhes(test)]/Number of writhes(control) [9].

RESULTS

The fluorescence analysis of powdered flower material was subjected to analysis under long ultra violet light after treatment with various chemical and organic reagents. The fluorescence's behavior was noted as in table-1

Table 1: Fluorescence study with different chemical reagents in visible and UV light of *Blumea mollis* powder

S. No.	Particulars	Visible	UV (254nm)	UV (365nm)
1	Powder as such	Green	Dark Green	Dark Green
2	Powder + distilled water	Green	Green	Dark Green
3	Powder+1 N NaOH in distilled water	Greenish Yellow	Dark Green	Dark Green

4	Powder+1 N NaOH in alcohol	Green	Dark Green	Greenish Brown
5	Powder+10% HCl	Light brown	Brown	Caramel brown
6	Powder+Conc. HCl	Brown	Greenish Black	Dark Green
7	Powder+Conc. HNO3	Brown	Wood Brown	Black
8	Powder+Conc. H2So4	Yellowish Green	Dark Green	Brownish Black
9	Powder+5% FeCl3	Yellowish Green	Brownish Black	Black

Table 2: Analgesic activity (Hot plate model)

Group	**Treatments**	**Dose(mg/ kg)**	**Reaction time in seconds at time (minutes) (mean±sem)**				
			0 min	**30 min**	**60 min**	**90 min**	**120 min**
G-I	Normal saline	1ml	1.22±0.34	1.22±0.06	1.31±1.66	1.33±.06	1.20±0.03
G-II	Indomethacin	50 mg/kg	6.23±.020	21.32±1.16	23.12±.27	2.01±.14	1.88±.14
G-III	Extract of *Blumea mollis*	100 mg/kg	5.14±0.12	12.16±0.12	10.14±0.08	1.92±0.12	1.89±0.08
G-IV	Extract of *Blumea mollis*	200 mg/kg	4.12±0.14	18.16±0.33	16.12±0.32	1.16±0.21	1.12±0.18
G-V	Extract of *Blumea mollis*	400 mg/kg	5.14±0.22	20.14±1.08	22.12±0.08	1.82±0.08	1.62±0.12

Table 3: Analgesic activity (tail flick/immersion model)

Group	**Treatments**	**Dose/(mg/kg)**	**Reaction time in seconds at time (minutes) (mean±sem)**				
			0 min	**30 min**	**60 min**	**90 min**	**120 min**
G-I	Normal saline	1ml	6.04±0.21	6.02±0.12	6.12±0.02	6.20±0.06	5.18±0.18
G-II	Indomethacin	50 mg/kg	4.02±0.04	5.04±0.02	26.12±0.02	21.06±0.08	6.08±0.12
G-III	Extract of *Blumea mollis*	100 mg/kg	5.04±0.02	6.04±0.12	8.02±0.16	8.04±0.06	6.03±0.14
G-IV	Extract of *Blumea mollis*	200 mg/kg	4.08±0.06	18.16±0.12	21.30±1.14	11.12±1.02	8.04±0.16
G-V	Extract of *Blumea mollis*	400 mg/kg	4.04±0.16	24.12±0.12	25.12±0.08	20.02±0.18	12.14±0.12

STATISTICAL ANALYSIS

The data was summarized using the mean and standard deviation. Statistical analysis was performed with GraphPad Prism 9.3.1 software, utilizing one-way Analysis of Variance (ANOVA) and the Newman–Keuls test to assess all data. A significance level of ($p < 0.001$) was deemed statistically significant.

DISCUSSION

The plant *Blumea mollis* belonging to family Asteraceae used in various traditional system of medicine. The presence diverse categories of bioactive compounds in *Blumea mollis* indicated the medicinal potential of *Blumea mollis* these bioactive constituents are multifunctional natural products acting through multiple targets by being analgesic. It is well known that pharmaceutical companies around the world are interested in developing safer and more effective drugs to treat pain. The present study evaluated the significant analgesic effect of ethanolic *B.mollis* extract in animal model.

To investigate for analgesic activities of the extract of *B.mollis*. we used tail flick methods. Intraperitoneal administration of acetic acid (0.8%) caused localized inflammation in due rats to the biogenesis of prostaglandins and leukotrienes. The biosynthetic prostaglandins, particularly prostacycline and prostaglandin E, have been reported to be responsible for the pain sensation due to intraperitoneal administration of acetic acid (10). Diclofenac sodium like other non-steroidal anti-inflammatory drugs, inhibits the biogenesis of prostaglandins, thus inhibiting the writhing in experimental animals like Rats. As our extract of *Blumea mollis* showed significant analgesic activity in thermal heat method, it can be assumed that the extract could act by a central anti-nociceptive mode like that of pentazocine. In the present study, we investigated analgesic activity of three doses of ethanolic extract of *Blumea mollis* [11,12,13]. The study indicated that at doses of 200mg/kg body weight the ethanolic extracts caused significant . Results of three doses were also comparable with those of standard drug. Similar analgesic activities were also observed in tail immersion and tail flick test. Doses of the seed extract significantly and dose dependently increased the elongation of tail flicking time in rats when compared to standard drug. ethanol exhibits significant analgesic effect in albino rats. We believe that further detailed advanced studies may be pursued in the future to explore the analgesic activities of the plant as well as its active constituents. The results of this study exhibited that ethanolic *B. mollis* extract possesses analgesic activities.

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