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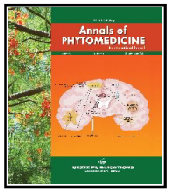

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Fractionation and antidepressant activity of hydroalcoholic extract fraction of *Celosia cristata* L. leaves

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Abstract

Traditional medicine uses *Celosia cristata* L. (Amaranthaceae) to treat several ailments. It belongs to the Celosia genus and is referred to as cockscomb because the blossom resembles the head of a rooster. Different plant sections were used to isolate a number of essential compounds. In view of the antidepressant activity of hydroalcoholic extracts of leaves and flowers of *C. cristata* in albino wistar rats by forced swim test noted in prior research, the crude hydroalcoholic leaf extract of *C. cristata* was fractionated by the funnel method to yield six fractions. But, the petroleum ether fraction was to produce no yield. The bioactivity of the five fractions obtained was evaluated for antidepressant activity in albino wistar rats by forced swim test. The results of the effect of various fractions, *i.e.*, chloroform, acetone, ethyl acetate, methanol, and water upon treatment once daily orally for 14 days on the immobility time (seconds) in the forced swim test resulted in a decrease in immobility time in rats. It was noted that there was a significant reduction (p<0.05) in immobility time in group methanolic fraction (MEF 400 mg/kg) treated rats when compared to stress-induced rats, showing their potent antidepressant action.

1. Introduction

Nature has long been a source of essential therapeutic compounds, and a remarkable number of contemporary medications have been identified and manufactured from plant sources. The use of herbs and plant medicines in the treatment of human illnesses is becoming a worldwide phenomenon, and it has recently gained enormous popularity and recognition as a result of substantial validation from scientific research. Every civilization in the world has depended on the diverse natural chemistry contained in plant medicines for medicinal purposes, whether through written or oral history (Otshudi *et al.,* 2000). Depending on the community, a single plant or a mixture of two or more plants may be used to cure a variety of illnesses. Plants and their derivatives have been used to cure neurodegenerative and chronic disorders (Gezici *et al*., 2020). Traditional medicinal herbs have been used successfully to treat a variety of diseases, including those that affect the heart, lungs, blood pressure, rheumatoid arthritis, sickle cell illness, fever, some types of cancer, and infectious diseases of the urinary and respiratory tracts (Cousins *et al.,* 2002; Saganuwan, 2010). Millions of people throughout the world suffer from depression, a serious mood disease. In addition, according to the World Health Organisation, depression is the fourth most common cause of illness globally, after only lower respiratory infections, heart diseases, prenatal disorders, and HIV/AIDS (Sutar *et al*., 2021). Two-thirds of people who are depressed have suicidal thoughts, and 10-15% of those people try to take their own lives. The monoaminergic transmitters noradrenalin, 5-hydroxytryptamine, and dopamine are functionally deficient in the brain, which is the primary cause of depression's major symptoms (Meyers, 2000; Quadri *et al*., 2021). The central nervous system (CNS) levels of these neurotransmitters are raised by medications that have antidepressant properties. The main antidepressant treatments work to normalize neurotransmission by increasing the amount of transmitters in the neurons (Jithan and Chinnblaiah, 2009). Many of the antidepressant medications that are now on the market have proven to be helpful, but they come with drawbacks, including a variety of side effects, troublesome interactions, and a very low response rate (Tamminga *et al.,* 2002). Just two out of three patients are said to respond to a particular treatment, and of those, one would have likely responded to a placebo by itself. Drugs derived from natural sources often have high efficacy, minimal danger, and few adverse effects. The pursuit of innovative plant-based medication for mental diseases has advanced greatly in recent years. Herbal treatments should thus be viewed as supplemental or alternative medications (Jintanaporn *et al.,* 2007). The recommended method for examining the phytochemical makeup and activity of herbal extracts is fractionation, particularly when those extracts are derived from plant varieties that are commonly used in traditional healthcare and may be essential sources of metabolic products with advantageous effects on human wellness (Sierra-Rivera *et al.,* 2021). One such plant is *C. cristata*, a flower that is regarded as one of the best therapeutic plants in the Kashmir valley. *C. cristata* is an herbaceous plant in the family Amaranthaceae (Caryophyllales). It is a variety of *C. argentea*. Because of its beautiful and vibrantly colored inflorescences, it is planted as an ornamental plant across much of the world. Its leaves and flowers are used as vegetables in several regions of the world, including Africa, India, China, Indonesia, and other regions of Asia. The plant was discovered to contain phenolic chemicals, flavonoids, tannins, and sterols in general. Numerous writers have written papers on the detection and isolation of various chemicals from plant seeds

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and leaves. These include 4-hydroxy-phenethyl alcohol, stigmasterol, -sitosterol, and 2-hydroxyoc-tadecanoic acid. Saponins (such as cristatain, celosin A, B, C, and D, and semenoside A) and glycoproteins (CCP-25 and CCP-27) are also present (Wang *et al.,* 2010). According to reports, dried flowers, leaves, and seeds are employed in Chinese, African, and Indian (Ayurvedic and Unani) traditional medical practices to treat a variety of illnesses. Different portions of *C. cristata* are employed by conventional healers for ethnomedicine. The dried red blossoms have antihematuric, antiabdominal, antileucorrheal, antihemorrhagic, antihemoptysis, antiosteoporosis, and antiheavy menstrual bleeding properties. In addition to its traditional usage in medicine, scientific studies have revealed its hepatoprotective, antibacterial, anthelmintic, antinociceptive, antioxidant, preventing ageing, and adipogenesis reduction effects. Most of the traditional healers (hakims) in the Kashmir valley employ the Unani system of healing and, depending on the condition being treated, use dried, powdered red flowers and seeds of *C. cristata* in precisely calculated quantities (Sayeed *et al.,* 2020). One essential method for isolating chemicals from medicinal plants with high biological activity is fractionation. The objective of the current work was to fractionate *C. cristata* leaves hydroalcoholic extract (CCLHAE) and look into its antidepressant properties. This present study sought to separate CCLHAE into fractions and investigate its antidepressant activity.

2. Materials and Methods

2.1 Plant material

In the month of February 2021, *C. cristata* leaves were taken from a rural region of Gwalior (M.P.). Dr. Arti Garg, Senior Botanist, Scientist-E, and Head of Office, Botanical Survey of India, Central Regional Centre, 10 Chatham Lines, Allahabad-211002, recognised the sample. A plant herbarium was sent to the specimen section of the Botanical Survey of India, Central Regional Centre, 10 Chatham Lines, Allahabad. The specimen voucher number for *Celosia argentea var. cristata* (L.) Kuntze is 2020-21/429, and the accession number is 105479. Plant matter was dried in the shade. With the use of a manual grinder, it was ground into a coarse powder. The coarse powder was sealed in an airtight container and kept in a dry and cool place. The material was used to continue the investigations.

2.2 Chemical reagents

In this investigation, imipramine hydrochloride (Sigma-Aldrich, St. Louis, USA) was used. All medications were mixed in distilled water and given orally or intraperitoneally (i.p.). All other chemicals and reagents were of analytical quality and were acquired from S.D. Fine Chemicals Pvt. Ltd., Mumbai, India, and SRL Pvt. Ltd. (Mumbai, India). Distilled water was used as the vehicle.

2.3 Animals

Albino wistar rats were used in this study. The Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI), Bhopal, authorised all animal research (CPCSEA Reg. No. 1824/PO/RcBi/S/15/CPCSEA). Protocol Approval Reference Number is PBRI/IAEC/29-03-22/009.

2.4 Soxhlet extraction

A powdered *Celosia cristata (C. cristata*) leaves were placed in a thimble of Soxhlet apparatus. Organic solvents were used for the extraction, including petroleum ether and 70% ethanol, which were applied for 8-10 h at a temperature of 40-60°C. After the extraction process, the extracts of the sample were filtered and concentrated to dryness. Extracts were collected in airtight containers (Dutta *et al.,* 2020; Bhuvaneswari *et al*., 2022). The extraction yield of all extracts was calculated using the following equation below:

Yield (%) = Actual yield/ Theoretical yield × 100

In the previous research, we concluded that hydroalcoholic leaves and flower extracts of *C. cristata* show a significant antidepressant activity as compared to petroleum ether extract (Tripathi and Khan, 2023).

2.5 Fractionation

Plant extracts are separated into different fractions throughout this procedure. Different solvents should be used in the sequence of increasing polarity when they are needed for fractionation.

2.5.1 Separation funnel method

When six distinct solvents are used (petroleum ether, chloroform, acetone, ethyl acetate, methanol, and water), the crude extract is moistened or completely dissolved in 250 ml of water to start the fractionation process. Then, the material is transferred into a separating funnel, shaken, and left to settle. Additionally, the least polar solvent was added to 250 ml of petroleum ether and shaken. The material can settle, and the bottom of the separating funnel can be opened to remove the aqueous layer. To get the petroleum ether fraction, the leftover material in the separating funnel was transferred into a fresh container. Another equal volume of petroleum ether was added, shaken, and separated. When no appreciable amount of extract seemed to be moving into the petroleum ether section after adding petroleum ether and shaking, the addition process was stopped. To obtain fractions of chloroform, acetone, ethyl acetate, methanol, and water, similar cycles were carried out. Due to the fact that the crude extract was initially dissolved in water, the leftover component after fractionation is known as the residual aqueous fraction (RAF).

2.6 Antidepressant activity

2.6.1 Forced swimming test

An evaluation tool for prospective antidepressant-like drugs is the forced swim test (FST), a rodent behavioral paradigm. The rat will be kept in a Plexiglas tank in this model. Water is added to the tank, and the rat's efforts to escape are scored. The rat strives to emerge from the water; this is referred to as mobility. The rat may eventually give up and become completely immobile, which is known as immobility, as its struggle movements eventually slow down. A decrease in the amount of time spent immobile throughout the testing period is referred to as antidepressant activity. The duration of immobility is assessed during the testing session. This technique was developed based on studying the behavior of animals forced to swim, in which they produce just the motions necessary to maintain their heads above the water before becoming inert and immobile after a time of intensive activity (struggling). After a few minutes of ferocious attempts to leave the water bath, each animal submitted to the testing circumstances and adopted a characteristic immobile posture with sporadic attempts to get away (Porsolt *et al.,*1979).

2.6.2 Experiment design

Rats were segregated into seven groups of six animals each.

Table 1: Groups of different solvent fractions at a given doses

Group	Sample	Dose
I	Negative control (untreated stress induced)	(untreated stress induced)
II	Imipramine standard	(30 mg/kg, i.p.) for 14 days
III	Chloroform fraction (CF)	400 mg/kg, in dw, p.o. for 14 days
IV	Acetone fraction (AF)	400 mg/kg, in dw, p.o. for 14 days
V	Ethyl acetate fraction (EAF)	400 mg/kg, in dw, p.o. for 14 days
VI	Methanol fraction (MF)	400 mg/kg, in dw, p.o. for 14 days
VII	Water/Aqueous fraction (AQF)	400 mg/kg, in dw, p.o. for 14 days

For a total of 14 days, treatment groups received one daily administration between 1 and 3 p.m. The rats were permitted to swim for 15 min during a trial session/pre-test session that was conducted following a 14-day treatment period, in accordance with the aforementioned equipment. The animals underwent a repeat exposure to the identical settings for 5 min 24 h following the pre-test session (test session). Drug solutions were given orally three times between the pretest session and the main session, as follows: immediately following the pre-test session, 5 h before the main test, and 1 h before the main test. When a rat floats in the water unharmed and uses just the barest minimum of leg movements to maintain its head above the surface, it is said to be immobile. Between 1 and 3 p.m., the forced swim test (FST) was conducted for 5 min. The time spent immobile was measured in seconds. The length of immobility throughout the FST was reduced, and this was used to evaluate the antidepressant's effectiveness (Sakakibara *et al.,* 2006; Hsu *et al.,* 2020).

3. Results

Nature provides us with the majority of the useful chemicals that directly benefit human existence and serves as an inspiration for brand-new synthetic or semi-synthetic structures that are essential for advancing both technical progress and living quality. Due to its availability, plant material has been the subject of the greatest research into natural drugs. In this experimental work, fractionation was done for CCLHAE (*Celosia cristata* leaves hydroalcoholic extract) solvent extract as it is showing the best *in vivo*. This sample is further fractionated in six solvents, but the petroleum ether fraction produced no yield, and the obtained yield will be maximum in methanolic and aqueous solvents (Figure 1). Thus, the yield obtained in the fractionation assay was employed in a further FST study.

Figure 1: Yield obtained in different solvent fractions.

The solvents fraction of CCLHAE studied for antidepressant effect using FST model. The results of each fraction were tabulated below.

Table 2: Effect of solvent fractions on immobility time using forced swim test

Groups	Treatment	Immobility time (sec)
I	Negative control (untreated stress induced)	202.30 ± 4.227
II	Imipramine standard (30 mg/kg, i.p.) for 14 days	88.20 ± 3.656**
III	CF 400 mg/kg, in dw, p.o. for 14 days	140.70 ± 4.412**
IV	AF 400 mg/kg, in dw, p.o. for 14 days	152.70 ± 6.593**
V	EAF 400 mg/kg, in dw, p.o. for 14 days	129.50 ± 2.811**
VI	MEF 400 mg/kg, in dw, p.o. for 14 days	110.50 ± 4.416**
VII	AQF 400 mg/kg, in dw, p.o. for 14 days	137.00 ± 4.195**

Values are expressed as Mean ± SD (n = 6); **p< 0.05 is statistically significant as compared to the stress-induced group by one-way ANOVA followed by Bonferroni's test. p>0.05 was considered as non-significant (NS) v/s stress induced.

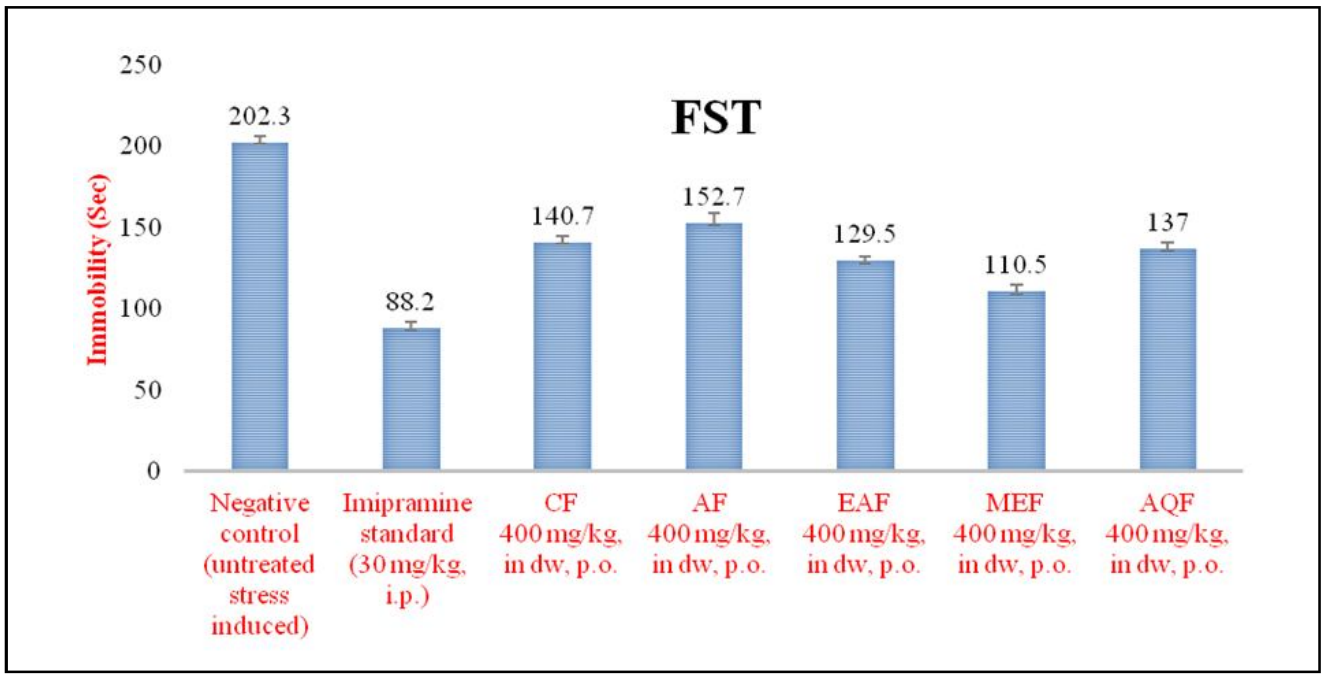

Figure 2: Immobility time at different solvent fractions by forced swim test (FST).

The results of the effect of various fractions chloroform fraction of *i.e.*, (CF), acetone fraction (AF), ethyl acetate fraction (EAF), methanol fraction (MEF), and aqueous fraction (AQF), upon treatment once daily orally for 14 days on the immobility time (seconds) in forced swim test resulted in reduction in immobility time in rats as shown in (Table 2). It was noted that there was significant reduction (p<0.05) in immobility time in group (MEF 400 mg/kg) treated rats when compared to stress induced showing their potent antidepressant action.

4. Discussion

The hydroalcoholic extracts of *C. cristata* leaves show a normal result of activity. Different solvent systems have been selected for the fractionation of the hydroalcoholic leaf extract of *C. cristata*. The solvent systems have been selected according to polarity, from non-polar to polar solvents. Six solvent systems have been selected for fractionation: petroleum ether, chloroform, acetone, ethyl acetate, methanol, and water. And in these six solvents, only five solvent systems give the fraction yield: chloroform fraction (CF), acetone fraction (AF), ethyl acetate fraction (EAF), methanol fraction (MEF), and aqueous fraction (AQF). The immobility times of the solvent extracts of CF, AF, EAF, MEF, and AQF at a dose of 400 mg/kg/day on the 14th day were found to 140.70 ± 4.412, 152.70 ± 6.593, 129.50 ± 2.811, 110.50 ± 4.416, and 137.00 ± 4.19 sec. The group treated with imipramine showed good activity at 88.20 ± 3.656 sec. There was no sign of any abnormality in the rats. The methanolic fraction (MEF) showed an antidepressant effect in the FST because it significantly reduced the immobility time compared with the vehicle-treated group. The group treated with imipramine showed good activity (88.20 ± 3.656 sec). The methanolic fraction (MEF) upon treatment once daily orally for 14 days on the immobility time (seconds) in the forced swim test resulted in a decrease in immobility time in rats.

5. Conclusion

The findings of this study provide the first details on the antidepressant properties of *C. cristata* leaves. When evaluated in models for the forced swim test, every fraction exhibited antidepressant activity. The methanol fractions showed the greatest reduction in immobility time ($p< 0.05$) among all the fractions when compared to stress-induced immobility, demonstrating their strong antidepressant activity. Alkaloids, flavonoids, saponins, and tannins were discovered to be present in the crude extract. The methanol fraction's phytoconstituents, which were enriched in the fraction as a result of fractionation, had the strongest antidepressant activity. Overall, the results of this investigation supported the hypothesis that the many phytochemicals found in plant extracts are probably what give them their pharmacological and therapeutic effects.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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