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Research Article

Pharmacological and Phytochemical Evaluation of *Calendula officinalis* Linn. For Anti-Anxiety Activity

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ABSTRACT

The Plant *Calendula officinalis* Linn. (Asteraceae), traditionally was found to be used in the treatment of anxiety. Despite a long tradition of use, no systematic pharmacological and phytochemical work has been carried out on this plant. Thus, *C. officinalis* was subjected to preliminary anti-anxiety screening studies, with a view to ascertain the truth on evidence of its traditional use as anti-anxiety. In the present study, aerial parts of the plant were extracted using solvents in the order of increasing polarity, viz., petroleum ether (60–80°C), chloroform, methanol and distilled water. Elevated plus maze (EPM) was used to evaluate the anti anxiety activity on all the crude extracts on mice. Methanolic extract of *C. officinalis* at the dose of 100 mg/kg, p.o. was found significant when compared to diazepam, (2mg/kg). Phytochemical screening showed the presence of alkaloids and polyphenols therefore specific methods are used to extract the total alkaloids and polyphenols fractions from the plant material and methanol extract. Polyphenol fraction at the dose of 50 mg/kg, p.o. showed significant anti-anxiety activity.

Keywords: *Calendula officinalis*, Anti-anxiety, Elevated plus-maze, Asteraceae.

INTRODUCTION

Calendula officinalis Linn. (Asteraceae), is a native plant of Mediterranean countries but is now grown as an ornamental plant throughout the world[1], and traditionally known as Pot marigold. It is an important medicinal plant used in our Traditional Systems of Medicine for treating various diseases like inflammations of internal organs, gastrointestinal ulcers, dysmenorrheal, as a diuretic, diaphoretic in convulsions, fever, and in cancer[2].

Calendula is an annual herb that grow fast, easy to germinate and simple to care. The genus name originated from the Latin *calendae* which means "first day of the month"[3].

It has been reported to possess many pharmacological activities, which include antioxidant[4], anti-inflammatory[5], antibacterial[6], antifungal[7] and antiviral[8]. It also possess cytotoxic as well as tumor reducing potential[9]. It is used as analgesic, anthelmintic, anti-bacterial, anti-emetic, anti-fungal, anti-inflammatory, anti-pyretic, antiseptic, anti-spasmodic, anti-viral, astringent, bitter, candidicide, cardiotonic, carminative, cholagogue, dermagenic, diaphoretic, diuretic, hemostatic, immunostimulant, lymphatic, uterotonic, and as vasodilator.

In Europe, the leaves are considered resolvent and diaphoretic while the flowers are used as a stimulant, antispasmodic and emmenagogue. In England, the decoction of the flowers was used as a posses drink for the treatment of measles and smallpox, and the fresh juice as a remedy for jaundice, constipation and suppression of menstrual flow. In India, the florets are used in ointments for treating wounds, herpes, ulcer, frostbite, skin damage, scars and blood purification. The leaves, in infusion, are used for treating varicose veins externally. Mother tincture of *Calendula* is used for the treatment of mental tension, insomnia and as nervine tonic[10-12].

Phytochemical reports on *C. officinalis* indicate that the plant contains flavonoids, alkloides, carotinoids, coumarines, quinones, glycosides and sterols. It also contains volatile oil, lupeol, quercetin, protocatechuic acid, amino acids and triterpinoids[2].

The literature search suggests that the *calendula* is a popular remedy for a variety of ailments and is one important ingredient in a number of Ayurvedic and Homeopathic medicine systems, still efforts are needed to verify its efficacy through scientific screenings in animal models.

MATERIALS AND METHODS

Plant material

Dried aerial parts of *C. officinalis* were procured from K. R. Indo German American Trading Company, Kurukshetra (Haryana), India in the month of November. Identity of the plant was confirmed through Dr. H. B. Singh, Scientist F, Head of Raw Material Herbarium and Museum (RHMD), National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India.

Animals

Laca mice (either sex; 20–25 g, n=5) were used in the present study. The animals were maintained on standard

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Table 1: Yield of various extracts of *C. officinalis* aerial parts.

Extract	Yield (% w/w)
Petroleum ether	5.26
Chloroform	7.42
Methanol	12.92
Water	19.35

environmental conditions and fed with standard rodent diet (Kissan Feeds Ltd, Mumbai, India) and tap water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee and care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India.

Drugs and preparation of the extract

Dried aerial parts of *C. officinalis* were powered and extracted with Petroleum ether (60–80ºC), chloroform (CHD, Mumbai) and methanol (S.D. Fine Chemicals Pvt.), distilled under normal atmospheric pressure. Four different extracts were prepared at three different concentrations (100 mg/kg, 200 mg/kg, 400 mg/kg).

Vehicle and standards

Distilled water and tween 80 (5%) was used as vehicle for preparing the various test doses of different extracts. Diazepam (2 mg/kg) (Ranbaxy Pharmaceuticals) used as standard drug for the anti-anxiety activity.

Elevated plus maze (EPM) model

The EPM apparatus consisting of two open arms (16 × 5 cm) and two closed arms (16 × 5 × 12 cm) extending from a central platform (5 × 5 cm) and were elevated to a height of 25 cm above the floor was used to observe anxiolytic behaviour in animals[13,14]. Each mice was placed at the centre of the EPM with its head facing the open arms. During this 5 min experiment, the behavior of the mice was recorded as: (a) the number of entries into the open arms, (b) average time spent by the mouse in the open arms (average time = total time spent in open arms/number of entries in arms). Extracts were administered orally using a tuberculin syringe fitted with oral canula. The dose administration schedule was so adjusted that each mice was having its turn on the EPM apparatus 60 min after the administration of the dose. During the entire experiment, the animals were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of EPM could invoke anxiety in the animals. Every time before placing each animal, the arena was washed with 5% alcohol to eliminate the possible bias due the odor left by the previous animal.

Phytochemical analysis

For the detection of phytochemical constituents various qualitative test were performed on all the four extracts[15].

Statistics

The results have been expressed as Mean ± Standard Error Mean (S.E.M). The test doses were compared among themselves, and also with standard and control by analysis of variance (ANOVA) followed by Student Neumann Keuls test[16]. Control group was also compared with the standard group.

Preparation of polyphenol and alkaloidal fractions of C. officinalis aerial parts

Aerial parts of *C. officinalis* were mixed with lime and extracted with chloroform. Then the extracted drug was further evaporated to obtain concentrate under reduced pressure. It was then partitioned in a separating funnel using 5×50 ml of 2% acidulated water (HCl-water). The aqueous fraction was basified using NaOH solution to pH 8-9 followed by partitioning with chloroform (5×50 ml). The chloroform fraction was rich in alkaloids (0.079% w/w). The bioactive methanol extract (25 g) of *C. officinalis* aerial parts was suspended uniformly in water, placed in three-necked round bottom flask connected with teflon stirrer, and partitioned with ethyl acetate by heating for 30 min at 50ºC with continuous stirring. This procedure was repeated five more times. All the shakings of ethyl acetate were concentrated under reduced pressure (6.752 g).

RESULTS

Table 1 shows the yield of various extracts and Table 2 shows results of phytochemical screening of various extracts of *C. officinalis* aerial parts. The mean time spent by the mice in open arms after oral administration of 100, 200 or 400 mg/kg of the extracts of *C. officinalis* aerial parts, diazepam (2 mg/kg) and the control (vehicle) has been shown in Table 3. Among the extracts tested, maximum anxiolytic activity was observed in the methanol at the dose of 100 mg/kg, p.o. Phytochemical screening showed presence of alkaloids and polyphenols in methanolic extract. Thus, specific methods were adopted to extract total alkaloidal fraction and polyphenol fraction from the plant material and methanol extract respectively. Both the fractions (25, 50 or 100 mg/kg, p.o.) were subjected for the evaluation of anti-anxiety activity using EPM in mice, shown in Table 4 compared to diazepam (2 mg/kg) and control (vehicle). At the dose 50 mg/kg of polyphenol fraction exhibited significant anti-anxiety activity, while alkaloidal fraction was devoid of activity.

DISCUSSION

Various extracts of *C. officinalis* (aerial parts) was evaluated employing a widely used anti-anxiety model i.e. EPM for the activity. This model is commonly used in animal anxiety activity because it is effective, cheap, simple, less time consuming, requires no prior training and does not cause discomfort to the mice while handling. The model is principally based on the observations that the exposure of animals to an elevated and open maze results in approach–avoidance conflict which is manifested as an exploratory-cum-fear drive. The fear due to height (acrophobia) induces anxiety in the animals when placed on the elevated plus-maze. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in motor activity, which is measured by the time spent by the animal in the open arms.

Dried extracts (petroleum ether, chloroform, methanol and water) of *C. officinalis* (aerial parts) was separately suspended in a suitable vehicle and administered orally to mice. The activity was compared with the control group as

Table 2: Results of phytochemical screening of various extracts of *C. officinalis* aerial parts.

Chemical test	*C. officinalis*			
	Pet. Ether extract	Chloroform extract	Methanol extract	Aqueous extract
Alkaloids	–	+	+	–
Coumarins	–	+	+	+
Flavonoids	–	-	+	-
Saponins	–	–	–	–
Sterols/triterpenoides	–	–	+	–
Carbohydrates	–	+	+	+
Tannins/polyphenols	–	+	++	–
Proteins	–	–	–	–
Amino acids	–	+	–	+
Fats/oil	-	–	–	–

+ : present, - : absent

Table 3: Anti-anxiety activity of various extracts of *C. officinalis* flowers using EPM.

Treatment	Dose (mg/kg)	Number of entries in open arms (Mean^n ± S.E.M)	Average time spent in open arms (Mean^n ± S.E.M)
Control	Vehicle	3.4 ± 0.47^a	28.7 ± 0.39^a
Diazepam (Standard)	2	8.3 ± 0.74*	36.7 ± 0.82*
Petroleum ether extract	100	2.7 ± 0.45^a	26.4 ± 0.34^a
	200	3.1 ± 0.77^a	27 ± 0.43^a
	400	3.5 ± 0.55^a	26.2 ± 0.24^a
Chloroform extract	100	2.5 ± 0.55^a	28.5 ± 0.44^a
	200	2.4 ± 0.55^a	28.1 ± 0.36^a
	400	2.9 ± 0.85^a	28.6 ± 0.45^a
Methanol extract	100	7.9 ± 0.89 *	36.9 ± 1.31*
	200	5.7 ± 0.85^a*	30.1 ± 1.25^a*
	400	3.8 ± 0.84^a	28.7 ± 0.73^a
Water extract	100	2.3 ± 0.45^a	28.3 ± 0.45^a
	200	2.8 ± 0.45^a	26.5 ± 0.51^a
	400	3.2 ± 0.77^a	26.3 ± 0.53^a

n=5; The data is expressed as Mean ± S.E.M; *P<0.05 vs Control; ^{a}P<0.05 vs Standard; ANOVA followed by Student Newmann Keul's test.

Table 4: Anti-anxiety activity of alkaloidal and polyphenol fractions of *C. officinalis* flowers using EPM.

Treatment	Dose (mg/kg)	Number of entries in open arms (Mean^n ± S.E.M)	Average time spent in open arms (Mean^n ± S.E.M)
Control	Vehicle	3.1 ± 0.45^a	29.1 ± 0.48^a
Diazepam (Standard)	2	7.8 ± 0.87*	37.2 ± 1.29*
Alkaloidal fraction	25	3.6 ± 0.35^a	29.2 ± 0.49^a
	50	3.1 ± 0.25^a	29.5 ± 0.36^a
	100	3.5 ± 0.75^a	29.4 ± 0.45^a
Polyphenol fraction	25	5.2 ± 0.83^a*	32.2 ± 0.63 ^{a}*
	50	7.6 ± 0.79*	36.9 ± 1.31*
	100	3.6 ± 0.52^a	29.8 ± 0.55^a

n=5; The data is expressed as Mean ± S.E.M.; *P<0.05 vs Control; ^{a}P<0.05 vs Standard; ANOVA followed by Student Newmann Keul's test.

well as with the group treated with the standard. Complete manifestation of anxiety in mice of the control group is evident from the minimum mean time spent in the open arms of EPM. Among the four extracts, methanolic extract exhibit maximum activity at the dose of 100 mg/kg in compared with that of diazepam which is evident from statistical equivalence.

Phytochemical screening showed the presence of alkaloids and polyphenols in methanolic extract of *C. officinalis* (aerial parts). Thus, specific methods were adopted to extract total alkaloidal fraction and polyphenol fraction from the plant material and methanol extract respectively. Both the fractions were subjected for the evaluation of anti-anxiety activity using EPM on mice at the doses of 25, 50 or 100 mg/kg, p.o. 50 mg/kg of polyphenol fraction

exhibited significant anti-anxiety activity, while alkaloidal fraction was devoid of the activity.

The studies are under progress to isolate bioactive constituents/fraction from plant responsible for anti-anxiety activity. Alkaloidal and polyphenol might be responsible for the anti-anxiety activity.

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