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

Rani Anita¹*, Mohan Chander²

¹Shiva Institute of Pharmacy, Bilaspur, Himachal Pradesh, India
²Rayat-Bahra Institute of Pharmacy, Hoshiarpur, Himachal Pradesh, India

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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 world¹, 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 cancer². *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 antioxidant⁴, anti-inflammatory⁵, antibacterial⁶, antifungal⁷ and antiviral⁸. It also possess cytotoxic as well as tumor reducing potential⁹. It is used as analgesic, anthelmintic, anti-bacterial, anti-emetic, anti-fungal, anti-inflammatory, anti-pyretic, antiseptic, anti-spasmmodic, anti-viral, astringent, bitter, candidicide, cardiotonic, carminative, chologogue, 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 nerve tonic¹⁰⁻¹².

Phytochemical reports on *C. officinalis* indicate that the plant contains flavonoids, alkaloids, carotinoids, coumarines, quinones, glycosides and sterols. It also contains volatile oil, lupeol, quercetin, protocatechuic acid, amino acids and triterpinoids². 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 diet and water ad libitum.
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 to 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. 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. 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 50°C 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

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

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

| Table 2: Results of phytochemical screening of various extracts of *C. officinalis* aerial parts. |
|-----------------------------------------------|-----------------------------------------------|
| Chemical test                           | Pet. Ether extract | Chloroform extract | Methanol extract | Aqueous extract |
| Alkaloids                                | -                 | +                  | +               | -              |
| Coumarins                                | -                 | +                  | +               | -              |
| Flavonoids                               | -                 | -                  | +               | -              |
| Saponins                                 | -                 | -                  | -               | -              |
| Sterols/triterpenoids                    | -                 | -                  | +               | -              |
| 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± S.E.M) | Average time spent in open arms (Mean± S.E.M) |
| Control                                    | Vehicle     | 3.4 ± 0.47* | 28.7 ± 0.39* |
| Diazepam (Standard)                        | 2           | 8.3 ± 0.74* | 36.7 ± 0.82* |
| Petroleum ether extract                    | 100         | 2.7 ± 0.45* | 26.4 ± 0.34* |
|                                           | 200         | 3.1 ± 0.77* | 27 ± 0.43*   |
|                                           | 400         | 3.5 ± 0.55* | 26.2 ± 0.24* |
| Chloroform extract                         | 100         | 2.5 ± 0.55* | 28.5 ± 0.44* |
|                                           | 200         | 2.4 ± 0.55* | 28.1 ± 0.36* |
|                                           | 400         | 2.9 ± 0.85* | 28.6 ± 0.45* |
| Methanol extract                           | 100         | 7.9 ± 0.89* | 36.9 ± 1.31* |
|                                           | 200         | 5.7 ± 0.85**| 30.1 ± 1.25**|
|                                           | 400         | 3.8 ± 0.84* | 28.7 ± 0.73* |
| Water extract                              | 100         | 2.3 ± 0.45* | 28.3 ± 0.45* |
|                                           | 200         | 2.8 ± 0.45* | 26.5 ± 0.51* |
|                                           | 400         | 3.2 ± 0.77* | 26.3 ± 0.53* |

n=5; The data is expressed as Mean ± S.E.M; *P<0.05 vs Control; **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± S.E.M) | Average time spent in open arms (Mean± S.E.M) |
| Control                                    | Vehicle     | 3.1 ± 0.45* | 29.1 ± 0.48* |
| Diazepam (Standard)                        | 2           | 7.8 ± 0.87* | 37.2 ± 1.29* |
| Alkaloidal fraction                        | 25          | 3.6 ± 0.35* | 29.2 ± 0.49* |
|                                           | 50          | 3.1 ± 0.25* | 29.5 ± 0.36* |
|                                           | 100         | 3.5 ± 0.75* | 29.4 ± 0.45* |
| Polyphenol fraction                        | 25          | 5.2 ± 0.83**| 32.2 ± 0.63**|
|                                           | 50          | 7.6 ± 0.79* | 36.9 ± 1.31* |
|                                           | 100         | 3.6 ± 0.52* | 29.8 ± 0.55* |

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