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Research Article

Evaluation of Anthelmintic Activity of *Calendula officinalis* Flowers Extract

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Abstract

Background: The present study is an attempt to explore the anthelmintic activity of ethanolic extract of flower of *Calendula officinalis*. Various doses of ethanolic extracts were evaluated for their anthelmintic activity on adult Indian earthworms, *Pheretima posthuma*. *Calendula officinalis* Linn has been widely used in homeopathic medicine for the treatment of many diseases. It has been reported to possess many pharmacological activities, which include antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory.

Methods: The ethanol extract of air dried flowers powder (500g) was prepared by using soxlet apparatus, concentrated and vacuum dried which gave an orange yellowish mass (60.46 g). Extract was subjected to qualitative chemical investigation of phytoconstituents such as glycosides, alkaloids, flavonoids, carbohydrates, tannins, proteins, vitamins, coumarins etc.

Results: All extracts were able to show anthelmintic activity at 10mg/mL concentration. The activities are comparable with the standard drugs, piperazine citrate and albendazole. The data were found statistically significantly by using one way Anova at 5% level of significance ($p < 0.05$).

Conclusion: All the doses of ethanolic extract of *calendula officinalis* showed dose dependent anthelmintic activity in comparison to standard drugs.

Keywords: *Calendula officinalis*, Asteraceae, Anthelmintic, Piperazine citrate, Albendazole

INTRODUCTION:

In recent years, the interest in folk medicine or Traditional Medicines from different cultures has increased significantly in modern countries, due to the fact that many prescription drugs worldwide have originated from the tropical flora.^{1, 2} Traditional plants have been used in many parts of the world long time ago where they offer a safe, cheap and reliable alternative to chemical drugs.^{3, 4} The nature has provided us various medicinal plants which became the storehouse of remedies to cure all ailments of mankind.⁵ India has a rich flora that is widely distributed throughout the country. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha.⁶

Calendula officinalis Linn has been widely used in homeopathic medicine for the treatment of many diseases. It has been reported to possess many pharmacological activities, which include antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory. It also possess cytotoxic as well as tumor reducing potential. It is used as anti-bacterial, anti-emetic, anti-fungal, anti-inflammatory, anti-pyretic, anti-septic, anti-spasmodic, anti-viral, astringent,

cardiotonic, carminative, cholagogue, dermagenic, diaphoretic, diuretic, hemostatic, immunostimulant, lymphatic, uterotonic, and as vasodilator. Externally it is used for treating skin inflammations, open wounds and laceration wounds with bleeding. It is also used for treating minor diseases like razor burns and wind burns. Internally it is used for mucous membrane inflammations, peptic and duodenal ulcers, spasms of the GI tract, duodenal and intestinal mucosa, dysmenorrhea (painful menstruation) especially in nervous or anemic women, splenic and hepatic inflammations. It is also used as a mouthwash after tooth extractions.⁷

Literature survey revealed that no systematic study has been reported for its anthelmintic activity. So, present study was carried out to find out the anthelmintic activity of the flowers of *Calendula officinalis*.

MATERIALS AND METHODS:

The flowers of *Calendula officinalis* were collected from Herbal garden of Jamia Hamdard, New Delhi. The sample was authenticated by Dr. H. B. Singh, Scientist Incharge, NISCAIR, New Delhi. A voucher specimen NISCAIR/RHMD/Consult/2008-09/1101/132 is preserved for future reference.

Preparation of the Extract: The ethanolic extract of air dried flowers powder (500g) was prepared by using soxhlet apparatus, concentrated and vacuum dried which gave an orange yellowish mass (60.46 g). Extract was subjected to qualitative chemical investigation of phytoconstituents such as glycosides, alkaloids, flavonoids, carbohydrates, tannins, proteins, vitamins, coumarins etc.

Preliminary phytochemical investigation of *Calendula officinalis* flowers

The ethanolic extract of flower was subjected to qualitative chemical investigation for the identification of different phytoconstituents like alkaloids, glycosides, sterols, saponins, carbohydrates, tannins, proteins, triterpenoids. Preliminary phytochemical screening was performed using the standard procedures.⁸ The presence of phytoconstituents such as alkaloids, glycosides, flavonoids, protein and amino acids, phenolic compounds, tannins, volatile oil and mucilage were confirmed as shown in Table 1.

Table 1: Preliminary phytochemical screening for the ethanolic extract of flowers of *Calendula officinalis*

S. No	Phytochemical test	Inference
1	Alkaloids	+
2	Glycosides	+
3	Sterols	-
4	Flavonoids	+
5	Saponins	-
6	Carbohydrates	-
7	Tannins	-
8	Resins	-
9	Proteins and Amino acids	+
10	Phenolic compounds and tannins	+
11	Mucilage	+
12	Volatile oil	+

+ = Present, - =Absent

Biological study: Healthy adult Indian earthworm (*Pheretima posthuma*), due to its anatomical and physiological resemblance with the intestinal round worm

parasites of human beings were used in the present study.⁹⁻¹¹ All earthworms were of equal size. They were collected from local place, washed and kept in water.

Drugs: The ethanolic extract of *calendula officinalis* was tested in various doses in each group. Normal saline water was used as control. Piperazine citrate and albendazole were used as standard drugs for comparative study with ethanolic extracts.

Experimental Method: The method of Pathak¹² was followed for screening of anthelmintic activity. Anthelmintic activity was evaluated on adult Indian earthworm, *Pheretima posthuma*. Earthworms were divided into nine groups (5 each). The first group (I) served as normal control which received saline water only. The second (II) and third (III) groups received the standard drugs piperazine citrate and albendazole at a dose level of 10mg/mL. Groups (IV) to (IX) received doses of ethanolic extracts of 10mg/mL, 15mg/mL, 20mg/mL, 25mg/mL, 30mg/mL, 35mg/mL respectively. Observations were made for the time taken to cause paralysis and death of individual worms for two hr. Paralysis was conformed when the worms did not revive even in normal saline water. Death was concluded when the worms lost their motility followed by fading away of their body colors.

Statistical analysis: The data on biological studies were reported as mean \pm standard deviation (n=5). For determining the statistical significance, standard error mean and analysis of variance (ANOVAs) at 5 % level significance was employed. P values <0.05 were considered significant.¹³

RESULTS:

The ethanolic extracts of *Calendula officinalis* produced a significant anthelmintic activity in dose dependent manner as shown in Table 2. Normal saline water was used as control. The activity shown by ethanolic extracts is of considerable importance. All data were found to be statistically significant at 5% level of significance ($P <0.05$) when subjected to one way ANOVA. The extent of activity shown by the crude extracts was found to be dose dependent and same effect as that of 10mg/mL of piperazine citrate was seen in 20-35mg/mL of ethanolic extract, and same effect as that of 10mg/mL of albendazole was seen in 25-35mg/mL of ethanolic extract as shown in Figure 1.

Table 2: Anthelmintic activity of ethanolic extracts of *Calendula officinalis*

Group	Treatment	Dose (mg/mL)	Time taken for paralysis (min) ($X \pm S.D.$)	Time taken for death (min) ($X \pm S.D.$)
I	Control (Normal saline)	----	----	----
II	Standard-1 (Piperazine citrate)	10	27.3 \pm 0.31	45.5 \pm 0.82
III	Standard-2 (Albendazole)	10	25.4 \pm 0.63	38.6 \pm 0.63
IV	Ethanolic extract	10	36.3 \pm 0.63	54.7 \pm 0.99
V	Ethanolic extract	15	33.7 \pm 0.02	49.5 \pm 0.85
VI	Ethanolic extract	20	28.5 \pm 0.42	42.7 \pm 0.09
VII	Ethanolic extract	25	26.1 \pm 0.56	34.4 \pm 0.76
VIII	Ethanolic extract	30	24.3 \pm 0.74	30.3 \pm 0.97
IX	Ethanolic extract	35	22.4 \pm 0.90	27.4 \pm 0.63

Each value is represented as mean \pm standard deviation (n=5), standard error mean < 0.492 , data are found to be significant by testing through one way ANOVA at 5% level of significance ($P < 0.05$).



Dead Earth Worm

Paralysed Earth worm

Figure 1: Anthelmintic studies against earthworms

DISCUSSION:

The anthelmintic activity of the ethanolic extract was comparable with that of standard drugs. Albendazole and piperazine are used as anthelmintic drugs, but these drugs show some unwanted effects like gastrointestinal disturbance, urticaria and bronchospasm and some patients experienced dizziness, paraesthesia, vertigo and in coordination. These drugs are also contraindicated in pregnant women and to those with compromised renal and hepatic function.

If we formulate anthelmintic drug from *Calendula officinalis* flowers probably this will show no unwanted effects, because the herbal formulations generally are safe, natural and have little or no side effects and as a raw material for the preparation of the formulation which is easy available with low cost. More work is needed to identify the main active principles which are responsible for the anthelmintic activity.

In order to assess the mechanism of action of the extract, its effect was tested on worms (*Pheretima posthuma*). Anthelmintic drugs like albendazole and piperazine citrate act by reversibly inhibiting neuromuscular transmission in the worm, probably by acting like GABA, the inhibitory neurotransmitter or GABA-gated chloride channels in nematode muscle that causes relaxation and depresses responsiveness to contractile action of Ach. Flaccid paralysis of the worms followed by death occurs.¹⁴⁻¹⁵ The fact that the extract of *Calendula officinalis* also shows paralysis in worms followed by death suggests that it may act like albendazole.

It could be concluded that the ethanolic extract of flowers of *Calendula officinalis* is having anthelmintic activity, compared to the standard drugs which is a significant result. Further studies are required to identify the actual chemical constituents that are present in the plant which are responsible for anthelmintic activity.

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