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Development, Formulation and Evaluation of Anthelmintic Property of *Tagetes erecta* Extract Loaded Suspension



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ABSTRACT

The present study was designed to develop, formulate and evaluate the *in-vitro* anthelmintic activity of different extracts of the stem of *Tagetes erecta* extract loaded suspension using different solvents like ethanol, water. Suspensions were prepared by using ethanolic and aqueous extract of the stem portion of *Tagetes erecta*. Prepared suspensions were evaluated by different parameters like sedimentation volume, pH, viscosity and particle size. The ethanolic suspension was found to be stable throughout the study and the values of different parameters was found to be in the optimum range such as sedimentation volume in the range of 97-99%, viscosity in the range of 50-54 cps, particle size in the range of 42-43 μ m and pH in the range of 6-6.7. The *In-vitro* anthelmintic activity of the suspension was carried out using adult earthworms *Pheretima Posthuma*. Various concentrations (150mg, 300mg) of the extract were tested by examining time taken for the paralysis and death of the worms. The time taken is noted and it was compared with Albendazole as standard reference and normal saline as control. The study revealed that ethanolic extract of formulated suspension of *Tagetes erecta* was more potent than Albendazole as the time taken for paralysis and death of the worms were lower in case of the ethanolic extract when compared to that of Albendazole.



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1.0 INTRODUCTION

Medicinal plants are being widely used all over the world and are becoming increasingly popular in modern society as an alternative to synthetic chemicals. During last few decades, there has been an increased growth in the usage of herbal medicines. It is getting popularized due to its natural origin and lesser side effects. The medicinal effects of the plant materials result from the combination of secondary products present in the plant. Plants produce many secondary metabolites as a protection against animals, parasites, bacteria and viruses and thus rely on these chemicals for their survival. These secondary metabolites provide the medicinal value of a plant, which in turn produces physiological action on the human body ⁽¹⁾. The WHO estimated that approximately 80% of world population relies mainly on these traditional medicines ⁽²⁾. Medicinal plants are a rich source from which antimicrobial agents are obtained. Plants are used in different countries for medicinal purposes and are a source of many potent and powerful drugs.

Marigolds are available in different colours, yellow and orange being the most common. Most of the *Tagetes* species have strong pungent odour and has a great value in cosmetic treatment. There are many varieties of *Tagetes* species available today. It is proved to have many pharmacological actions like antimicrobial, antioxidant, hepatoprotective⁽³⁾, insecticidal activities etc. It also contains many chemical constituents in which flavonoids ⁽⁴⁾ and lutein are the major ones.

Helminthiasis is prevalent in the world, particularly in third world countries. The overuse of chemicals for the management results in development of resistance. This led to the screening of medicinal plants for their anthelmintic activity ^(5, 6). Anthelmintic activity of a drug is their ability to expel parasitic worms from the body. The active constituents and secretions present in the plant are effective against nematodes.

Oral route of drug administration is most important for obtaining systemic effects. Parenteral route, except in few cases are not used routinely for self-administration of medicine. So, oral suspensions, solutions, syrups etc. are used for producing special effects of the medicinal agents ⁽⁷⁾.

The main objective of the work is to develop and evaluate the anthelmintic activity of *Tagetes erecta* (African or American variety) stem extract loaded suspension.

2.0 MATERIALS AND METHODS

2.1 Collection of plant materials: The fresh plants of *Tagetes erecta* were collected in the month of January (2018) from the plant growing near to Nehru College of Pharmacy, Pampady, Thrissur, Kerala, India. The plant was identified with the help of Zereena Viji, Assistant Professor of Botany department, NSS College, Nemmara and a voucher of specimen has been deposited at the botany department of university. Plant samples are dried by shade drying.

2.2 Preparation of extracts: The stem portion of the plant was shade and dried pulverized into fine powder using a grinder. About 50g of powdered material was extracted by maceration with 250ml of 95% ethanol and water for 14 days. The extract obtained with each of the solvent were filtered and the respective solvents were evaporated with the help of heating mantle. A sticky greenish brown substance was obtained from both ethanolic and aqueous extracts after solvent evaporation, and is stored in a refrigerator ⁽⁸⁾, which is then formulated into suspension.

2.3 Preparation of suspension

The composition for the formulation of suspension is shown in table 1. The samples of both the extracts are sufficiently triturated and made into fine particles. After that, the sample mixed with ethanol and water respectively. To each extract, the different additives such as sodium lauryl sulphate, sodium carboxy methyl cellulose, glycerine (humectant), sweetening agent and flavouring agent and preservative is added for its better stability during shelf life.

Table No. 1: Formulation of suspension

Sr. No.	Ingredients	Official Formula(g) (for 500ml)	Working Formula (150 mg)	Working Formula (300 mg)
1	<i>Tagetes erecta</i> stem extract (ethanol)	16	0.15	0.30
2	<i>Tagetes erecta</i> stem extract (aqueous)	16	0.15	0.30
3	Sodium lauryl sulphate	0.40	0.00375	0.0075
4	Glycerin	40	0.375	0.75
5	Methylparaben	0.80	0.0075	0.015
6	Propylparaben	0.16	0.0015	0.003
7	Carboxymethyl cellulose sodium	7	0.065	0.13
8	Saccharin sodium	0.40	0.0037	0.0075
9	Flavour	q.s	q.s	q.s

The composition given in the table is used for the formulation of suspensions of both ethanolic and aqueous extracts of the sample in this study.

2.4 Evaluation of suspension ⁽⁹⁾

Sedimentation volume: Sedimentation volume (F) is a ratio of the final volume of sediment (Vu) to the original volume of sediment (Vo) before settling. 50 ml of each suspension were transferred to 50 ml measuring cylinders and the volume of sediment formed was noted at every 24 hour for 7 days. The sedimentation volume F (%) was calculated using the formula:

$$F = 100 V_u / V_o$$

Viscosity measurement: The viscosity of the samples was determined using the Brookfield viscometer, at 30 rpm.

Particle size measurement: The particle size of the prepared suspensions was measured by microscopic method. The size of 100 particles was measured and the average particle size was determined.

pH: The pH of the prepared suspension was measured by using digital pH meter.

2.5 *In - vitro* anthelmintic activity:

The evaluation of anthelmintic property was performed on an adult earthworm *Pheretima Posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites in human beings ⁽¹⁰⁾. Earthworms were placed in a petri dish and two different concentrations each of ethanolic and aqueous extracts were poured and observed for its paralysis and death ⁽¹¹⁾. The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously, and death was concluded when the worms lost their motility followed with fading away of their body colour.

3.0 RESULTS AND DISCUSSION

Sedimentation volume of ethanolic and aqueous extract was found to be 98.38% respectively at the end of 24 hrs. The formulation of ethanolic and aqueous extract revealed good flow from container.

Table No. 2: Sedimentation volume

Formulation	Sedimentation volume (%)
Aqueous extract	97.23
Ethanollic extract	98.38

Viscosity of the ethanollic and aqueous extract was found to be 53.12cps and 49.23cps respectively.

Table No. 3: Viscosity

Formulation	Viscosity (cps)
Aqueous extract	49.23
Ethanollic extract	53.12

Particle size of ethanollic and aqueous extract was found to be 42.50and48.60 respectively.

Table No. 4: Particle size

Formulation	Particle size (µm)
Aqueous extract	48.60
Ethanollic extract	42.50

pH of ethanollic and aqueous extract was found to be 6 and 6.7 and respectively.

Table No. 5: pH

Formulation	pH
Aqueous extract	6
Ethanollic extract	6.7

Table No. 6: *In vitro* anthelmintic activity of various extracts of *Tagetes erecta* stem on adult earthworm (*Pheretima posthuma*)

Groups	Concentration used (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Control (normal saline)	25	-	-
	50	-	-
Standard (Albendazole)	25	41	49
	50	48	53
Suspension prepared with ethanollic extract of the sample	150	35	38
	300	30	34
Suspension prepared with aqueous extract of the sample	150	42	50
	300	39	47

By conducting further *in-vivo* studies, we can confirm that the stem extract of *Tagetes erecta* is more potent than Albendazole.



Control (Normal saline)

Figure No. 1: Control



Standard sample (Albendazole)

Figure No. 2: Standard sample



Suspension prepared with ethanolic extract of the sample

Figure No. 3: Ethanolic extract suspension



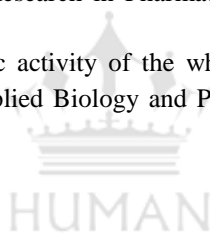
Suspension prepared with aqueous extract of the sample

Figure No. 4: Aqueous extract suspension

In the present investigation, it was observed that formulated suspension containing ethanolic extract of *Tagetes erecta* showed more potent anthelmintic activity. Anthelmintic activity of *Tagetes erecta* is confirmed by examining the time taken for paralysis and death of *Pheretima Posthuma*. It was observed that the ethanolic extract was more potent than the standard Albendazole. The formulated suspension was also evaluated for sedimentation volume, pH, viscosity, particle size and are found to be stable throughout the study.

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