



## PHYTOCHEMICAL INVESTIGATION OF *TAGETES ERECTA* FOR ANTI-DIARRHOEAL ACTIVITY

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### ABSTRACT

The object of the present investigation is to be screening of aqueous extract of *Tagetes erecta* (200 mg/kg and 400 mg/kg) administered by orally were selected on anti-diarrhoeal action. In this investigation, screening by castor oil induced diarrhoeal model, charcoal meal test model and PGE2 induced test model also dose of standard drugs is Loperamide (2 mg/kg) as well as atropine (0.1 mg/kg) were used in this study. Plant extract in dose of 200 and 400 mg/kg used followed by Phytochemical and acute toxicity study. In results of extract formed significant anti-diarrhoeal active against castor oil induced diarrhoea, charcoal meal test as well PGE2 induced diarrhoea. It also cause dose linked anti-diarrhoeal result. Results propose that it may proceed centrally and may inhibition of PGE2 to give anti-diarrhoeal action. Result of charcoal meal test also suggests its anti-muscarnic activity.

**KEY WORDS:** Diarrhoea, *Tagetes erecta*, Charcoal Meal, Castor Oil, Enteropooling.

### INTRODUCTION

Diarrhoea occurs in whole world and causes four percentages of all death and five percentages of strength defeat to disability. It is mainly usually cause by gastrointestinal infection which destroys approximately 2.2 million individuals global in every year, mainly infants in developing country. [1] Utilization of drinking water in sanitation is an important preventative criterion but infected water is too a significant reason of diarrhoea.

Cholera and dysentery cause strict, from time to time life intimidating forms of diarrhoea. The communicable agents that cause diarrhoea are there or are infrequently introduced all through the universe. Diarrhoea is an unusual incidence for mainly persons who exist in developed country where hygiene is extensively obtainable, entrance to secure water is soaring and individual and family hygiene is comparatively fine. Around the world 1.1 billion public need admittance to enhanced water resources and 2.4 billion have no basic hygiene. [2]

Diarrhoea due to illness is prevalent all over the developing nation. In Southeast Asia and Africa, diarrhoea is liable for as greatly as 8.5 % and 7.7 % of all deaths correspondingly. Every year around are about 4 billion belongings of diarrhoea international.

Diarrhoea is the transient of improved amount (greater than 300 g in 24 hours) of wobbly stools. It is commonly basis by a virus or bacteria and can be acute (short period of time) or chronic (huge period i.e. more than two or three weeks). Mainly persons are pretentious by diarrhoea at several times in their live. It is frequently accompanying by abdomen pain, emotion ill and sickness. It is frequently due to use of drinking water infected with bacterial, undercooked beef and eggs or insufficient kitchen hygiene-in other words, an illness.

### *Tagetes erecta* Leave

*Tagetes erecta* is belongs to genus of plant which were found in native to sub continents of Asia from Himalaya foothills of India to Sri-Lanka eastward through 20 Myanmar, Indonesia, Southern China and Hainan. *Tagetes erecta* have found to be grey colour bark, longitude striations on it and underneath it snowy bark is existent. Leaves are shape on bipinnately complex, 15-30 cm extended all manner 11-25 leaflets alternative on rachis, 2.5-3.5 cm extended ovate lanceolate with a slanting immoral. [3]

Margins occasionally creative, petioles 2-3 mm long, flowers are bisexual, white, funnel formed sweetly scented, stalked, whole, bracteates, regular with regular width of fully opened flower being in regular 1.12 cm inflorescence, incurable cymes each manner 60-90 flowers. Fruits are ovoid to subglobose, crumpled or uneven with glands. It is taking the size of 2.5 cm long and 0.3 cm in width and gets purplish dark when mature. Fruits are usually seeded. Seeds usually happen in spinach green colour which has eleven mm long and eight mm in diameter and weighs up to 445 mg. [4]

### Chemical Constituent

Leaves are aromatic and comprise proteins, carbohydrates, fiber, minerals, carotene, nicotinic acid and vitamin C. The leaves comprise chief constituents of oxalic acid, leaves also comprises crystalline glycosides, carbazole alkaloids, koenigin and resin. Fresh grasses cover yellow colour aromatic oil equally also rich in vitamin A and calcium. It's too contained Mahanimbicine, girinimbin, iso-mahanimbin, koenigine, koenine, koenidine and koenimbine. Bicyclomahanimbicine, phebalosin, coumarine as Murrayoneimperatoxinetc are elucidated from leaves. Tri terpenoid alkaloids like to be cyclomahanimbin and tetrahydromahanmbine are also presentence in the leaves. Murrayastine, murrayaline, pypayafoline carbazole alkaloids and all other phychemical compounds have been reported in the leaves of *M. koenigii*. In Bark chiefly founds carbazole alkaloids such as murrayacine, murrayazolidine, murrayazoline, mahanimbine, girinimbine, koenioline and xynthyletin. The tissue of fruits normally contained 64.9% moisture, 9.76% total sugar, 9.58% reducing sugar, 0.17% non-reducing sugar and negligible amount of tannin and acids. It should be containing 13.35% of vitamin C. The tissue of fruits comprises trace quantity of minerals 1.97% phosphorus, 0.082% potassium, 0.811% calcium, 0.166% magnesium and 0.007% iron. It also comprises makeable quantity of protein. [5]

### Use

The plant used anti-inflammatory, dysentery. The plants are accredited with tonic and stomachic assets. The root and leaves also used in anti-helminthic, analgesic, cure piles. Leaves are used in diarrhoea, dysentery and ulcer. [6]

## MATERIALS & METHODS

### Plant Authentication

The plants *Tagetes erecta* were authenticated by Dr. Zia-ul-Hasan, Department of Botany, Saifia College of Sciences and Arts, Bhopal (M.P.). A voucher specimen no. is Bio/saifia/2023/216.

### IAEC Approval for Animal Studies

In this study, wistar albino rats of either sex (male and female) weighing between 150 to 250 gm were used randomly. Institutional Animal Ethics Committee approves the experimental protocol; Proposal no. is CPCSEA/176/2023. Animals were maintained in standard circumstances in an animal house which has been approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Albino rats were used in this thesis was obtained from the RKDF College of Pharmacy, Bhopal. [7] The animals were in-housed in Poly propylene cages and maintain at  $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$  less than 12 hrs light / dark cycles and were nourish ad libitum with paradigm pellet diet and had liberated entrance to water. The animals were procured from standard diet supplied by Godrej Agrovet Ltd. Pune. The composition of the diet has been protein (10%), Arachis oil (4%), Fibers (1%), Calcium (1%), Vitamin A 1000 IU/gm and Vitamin D 500 IU/gm.

### Extraction of Plants

The leaves of *Tagetes erecta* were shadow dry and compact to coarse fine particles in a motorizeddicer. The powdered products obtain were then subjected to extraction in batchby various solvents in a soxhelet extractor. The different extracts found were evaporated at  $45^{\circ}\text{C}$  to get a semisolid mass. The extracts thus get were exposed to phyto-chemical study.

### Phytochemical Analysis of the Extracts

The extracts of plant were subjected to qualitative analysis for the various phytoconstituents like flavonoid, alkaloids, phytosterols, saponins, carbohydrates, glycosides, tannins, proteins, amino acids. [8]

### Acute Toxicity Study

The acute oral toxicity revise was agreed exposed for aqueous extract of the leaves of *Tagetes erecta* by fix dose means according to OECD rule no. 420. In this study Swiss albino mice (healthy adult female) weigh among 25 to 35 g. Animals were separated into four groups of five animals in each group and were reserved fast during the night. The various doses (5, 50, 300 and 2000) mg/kg b. w. were administered to the Group I, II, III, and IV correspondingly. [9] After that administering the extracts to various groups the behavioural change like body temperature, CNS action, micturation, defection etc were experiential for 24 h.

## EVALUATION OF ANTIDIARRHOEAL ACTIVITY Castor Oil Induced Diarrhoea

Rats of either sex (150-250gm) were fasted for 18 h. They were divided into four groups (n=6). The first group, which served as control was administered with aqueous 1% tragacanth suspension. The second group

received standard drug, Loperamide (2 mg/kg) orally as suspension. The extract was administered orally at 100 mg/kg dose to third group and 200 mg/kg dose to fourth group as suspension. After 60 min of drug treatment, the animals of each group received 1 ml of castor oil orally and the watery faecal material and number of defecations was noted up to 4 h in the transparent metabolic cages with filter paper at the base. Weight of paper before and after defecation was noted.

#### Charcoal Meal Test

Rats of either sex (150-200 g) were fasted for 18 h. They were divided into four groups (n=6). The first group which served as control was administered with aqueous 1% tragacanth suspension. The second group receives standard drug atropine (0.1 mg/kg) subcutaneously. [10] The extract was administered orally at 100 mg/kg to third group and 200 mg/kg to fourth group as suspension. The animals were given 1 ml of 10% activated charcoal suspended in 10% aqueous tragacanth powder p.o., 30 min after treatment. Animals were anaesthesia 30 min after charcoal meal administration by ether anaesthesia. The abdomen was cut off and the small intestine carefully removed. The distance travelled by charcoal plug from pylorus to caecum was measured.

#### PGE2 induced Diarrhoea

Rats of either sex (150-200 g) were fasted for 18 h. They were then divided into four groups (n=6). A solution of PGE2 was made in the 5%v/v in the normal saline. [11] The first group, which served as control, was administered with PGE2 (100 µg/kg p.o.) only. The second group, which served as vehicle control was administered with aqueous 1% tragacanth suspension by oral route. The extract was administered orally at 100 mg/kg to third group and 200 mg/kg to fourth group as suspension. Immediately after extract administration PGE2 was administered. After 30 min following administration of PGE2 each rat was sacrificed and whole length of the intestine from pylorus to caecum was dissected out, its content collected in measuring cylinder and volume measured.

#### Statistical Analysis

The data are represented as mean  $\pm$  S.E.M, and statistical significance between treated and control groups was analyzed using of ANOVA, followed by Tukey where  $P < 0.05$  was considered statistically different. [12]

## RESULTS AND DISCUSSION

### ACUTE TOXICITY STUDIES

The acute toxicity studies of the different plants were performed. Aqueous extract of *Tagetes erecta* (200 mg/kg and 400 mg/kg), orally was selected for different diarrhea induced models

Figure 1:



### PRELIMINARY PHYTOCONSTITUENTS:

Table 1: The preliminary phytochemical analysis of extract revealed that the presence of various phytoconstituents.

Constituents	Chemical test	<i>Tagetes erecta</i> Extract
Alkaloids	Hager's Test	+ ve
	Mayer's Test	+ ve

	Dragendroff's Test	+ve
	Wagner's Test	+ ve
<b>Carbohydrates</b>	Molish's Test	+ ve
	Fehling's Test	+ ve
	Benedict's Test	+ ve
<b>Cardiac glycosides</b>	Baljet's test	-ve
	Legal's test	+ve
<b>Anthraquinone glycosides</b>	Borntrager's test	+ ve
	Modified Borntrager's test	+ ve
<b>Saponin glycosides</b>	Foam test	+ve
<b>Fixed oil</b>	Stain Test	+ve
<b>Proteins and Amino acids</b>	Millon's Test	+ ve
	Biuret Test	+ ve
	Ninhydrin Test	+ ve
<b>Triterpenoids</b>	Liebermann-Burchard Test	+ ve
<b>Flavonoids</b>	Shinoda test	+ ve
	Sodium hydroxide test	+ ve
<b>Tannin and Polyphenols</b>	Lead acetate solution	+ ve
	5% FeCl <sub>3</sub> solution	+ ve
	Bromine water test	+ ve
	Potassium dichromate test	+ ve

**Table 2: Evaluation of anti-diarrhoeal activity of aqueous extract of *Tagetes erecta* by castor oil induced diarrhoea.**

Treatment	Total number of faces	Total number of diarrheal faces	Delay in defecation time (min)
Control	10.33±0.31	7.68±0.42	6.41±0.08
Loperamide (2 mg/kg)	5.18±0.18	2.48±0.17	2.76±0.17
Aqueous extract (200 mg/kg)	6.53±0.18	4.35±0.73	3.24±0.62
Aqueous extract (400 mg/kg)	5.80±1.09	4.20±0.20	3.35±0.14

Number of animals (N) =6, values are expressed as mean±SEM

**Table 3: Evaluation of anti-diarrhoeal activity of aqueous extract of *Tagetes erecta* by charcoal meal test.**

Treatment	Movement of Charcoal
Control	89.04 ± 0.16
Atropine sulphate (2 mg/kg)	18.48 ± 0.24**
Aqueous extract (200 mg/kg)	54.80 ± 0.82**
Aqueous extract (400 mg/kg)	71.64 ± 0.16**

Number of animals (N)=6, values are expressed as mean±SEM, \*\*=P<0.01= very significant.

**Table 4: Evaluation of anti-diarrhoeal activity of aqueous extract of *Tagetes erecta* by PGE2 induced enteropooling.**

Treatment	Volume of intestinal fluid (ml)
PGE2 control	3.1 ± 0.97
Vehicle control	2.98 ± 0.10
Aqueous extract (200 mg/kg)	1.89 ± 0.19**
Aqueous extract (400 mg/kg)	1.10 ± 0.11**

Number of animals (N) =6, Values are expressed as mean±SEM, \*\*=P<0.01= very significant.

## DISCUSSION

In research of Phytochemistry, Plant products and also its isolated constituents some of the chief gorgeous source of novel drugs, and have been shown promise effect for the action of diarrhoeal in various induced experimental models for evaluating anti-diarrhoeal agents. [13] The

systematic review of the Ayurvedic, traditional and scientific literature was carried out to select the plant. In the present investigation plants were selected that was used traditionally as ulcero-protective and anti-diarrhoeal but not screened for antiulcer activity and anti-diarrhoeal action.

The preliminary phytochemical evaluation plant extract shown the occurrence of flavonoids, saponins, tannins, alkaloids, and lipids. In qualitative analysis indicate that the aqueous and ethanolic extract is extremely wealthy in flavonoids, tannins, lipid and alkaloids. Tannins and flavonoids are identified as in nature occurring complex having gastro-protective and anti-diarrhoeal action. [14]

Aqueous extract was prepared and phytochemical analysis revealed presence of flavonoids and tannins as a major constituent. Many phytochemical constituents like flavonoids, tannins, terpenes that are reported to possess antiulcer and anti-diarrhoeal activity are also present in extract. [15] These phytochemicals have been proposed to explain their gastro-protective and anti-diarrhoeal effects by several mechanisms in the present study.

Every doses of plant extract shown active against PGE<sub>2</sub> induced enter pooling, which strength be owing to the reserve of mixture of prostaglandins. [16] Anti-enter pooling action of the extract is more pertinent since the avoidance of enter pooling help in the reserve of diarrheal. [17] Alcoholic and aqueous extract and the anti-muscarinic drug, atropine (0.1 mg/kg) decrease the propulsive group in the charcoal meal test. [18,20] The original mechanism appears to be spasmolytic and an anti-enter pooling action by which the extract formed release in diarrhoea.[21] Tannic acid and tannins are there in a lot of plants and they denature proteins form protein tannate complex. The complex created coat above the intestinal mucosa and make the intestinal mucosa extra opposed to and reduced is charge. [22] The tannin there in the plant extracts may be conscientious for the anti-diarrhoeal action.

## SUMMARY & CONCLUSION

Phytochemical analysis of the extract revealed that extract contains the Carbohydrates, flavanoid, saponin,

tannin/polyphenol and fat, phyto-sterols and tri-terpenoids. From the acute toxicity study of the hydro-alcoholic extract was establish to be non-lethal up to doses up to 2000 mg/kg body weight of the animals.

Anti-diarrhoeal action was screened by using Castor oil induced diarrhoea, charcoal meal assay and PGE<sub>2</sub> induced enter pooling assay. The extract of leaves *Tagetes erecta* exhibited pronounced anti-diarrhoeal effect in a dose-dependent manner following oral pre-treatment on castor oil-induced diarrhoea compare with the positive standard. The effect of extract increases the onset time of diarrhoea. Though the action is important but relatively it is smaller than the loperamide 2 mg/kg. The extract considerably (P < 0.01) reserved together the incidence of defecation as well as the wetness of the faecal dung of rat. Effect of plant extract on intestinal transit was observer using charcoal meal test. Distance travel by the charcoal meal were compact in the extract treat test groups with the dose of 100 mg/kg, 200 mg/kg and 400 mg/kg correspondingly, compare to control group. Anti-enteropooling action was assessing by using PGE<sub>2</sub> induce diarrhoea. The extract reserved PGE<sub>2</sub>-induced enter pooling notably (P<0.01) in mice at every dose. The anti-diarrhoeal result of it might also due to the occurrence of tannins and flavanoids in the extract and maybe which are accountable for the over property.

In conclusion, aqueous extract of *Tagetes erecta* possessed significant anti-diarrhoeal activity against different diarrhoeal induced models. The anti-diarrhoeal result of aqueous extract is outstanding to decrease of GIT motility, reserve of the synthesis of prostaglandin and NO. The extract has potential effect on the reduction of gastrointestinal motility than the other effects. The above effects of it may also be due to the presence of tannins and flavanoids in the extract.

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