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In vitro carminative and *in vivo* antidiarrheal activity of *Citrus maxima*

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ABSTRACT

In this study, the methanolic extracts of leaves of Citrus maxima were evaluated for their carminative and antidiarrheal activities. By soxhlet method, the methanolic extract of leaves of Citrus maxima was prepared. Carminative activity of methanolic extract was studied in vitro by acid-base titration technique and antidiarrheal activity was studied in rats by castor oil induced diarrhoea method. The phytochemical screening was done using various chemical tests. Alkaloids, tannins, carbohydrates, flavonoids, terpenoids, amino acids, anthraquinones, glycosides are confirmed to be present in the leaf. Carminative activity of extract was found significantly less with 5mL, when compared with 10mL extract. The anti-diarrheal activity observed in the animals treated with 200mg/kg showed significant activity compared with the animals treated with 100mg/kg. The study revealed that carminative activity and antidiarrheal activity of methanolic extract of *C. maxima*. May be the extract acts on circular and longitudinal intestinal muscle by inhibiting acetylcholine and prostaglandins, which reduces peristaltic movements and prolongs transmit time, reduces fecal volume, increases viscosity and viscous fluid and electrolyte loss. The compound in the extract causes mild irritation resulting in vasodilation of gastrointestinal muscles, thereby relieving cramping and expels gas.

Keywords: Carminative, Antidiarrheal, Malabsorption, Loperamide, Soxhlet apparatus.

INTRODUCTION

World Health Organization (WHO) statistics indicate that almost 80% of the world's population trusts traditional medicines for treating their health needs due to their greater cultural acceptability, less side effects, and adapts with the human body [1].

Shaddock or pomelo is typically referred to as Citrus maxima (Family: Rutaceae). It is aboriginal to tropical regions of Asia. In ancient and medieval literature, the pulp and fruit are mentioned as antitoxic, appetizers, cardiac stimulants, and stomach tonics. Recently, leaves are found to exhibit antitumor activity [2]. Additionally, alcohol extracted from the fruit has anti-diabetic and anti-hyperlipidemic properties [3].

The plant *C. maxima* has been previously evaluated for antiulcer activity, antioxidant activity, anti-arthritic and anti-inflammatory activity, antibacterial activity [4] and anticancer activity [5]. Due to excess production of HCl in the stomach, the mucous membrane protecting the inner layer of the stomach wall (made up of simple epithelium) will disintegrate and result in ulcer formation. This will also result in the formation of gas in the gut and leads to flatulence. Considering the antiulcer activity, have experimented to identify anti carminative activity. Since pomelo juice is bitter and astringent in nature, protein precipitation occurs so that the cell's external surface area lies impermeable to the passage of fluids, finally minimizing the water loss. Considering the astringent activity, we have screened for anti-diarrheal activity.

MATERIALS AND METHODS

Experimental animals

Male Albino rats (Wistar strain) weighing 150-200g were utilized in the study under standard conditions (24° C room temperature and 60 to 65% humidity). Pellets are the food (Rayan Biotechnologies Pvt Ltd) and water is available ad libitum. The ethics committee of the institute approved the animal experiments.

Chemicals and drugs

Methanol, sodium hydroxide, standard HCl, phenolphthalein, methyl orange, hydrochloric Acid, calcium carbonate, sodium carbonate, citrus maxima leave extract, distilled water, castor oil, loperamide (standard drug).

Preparation of methanolic extract

For seven days, *Citrus maxima* leaves were collected in the local area and dried under shade in a room and they were grounded into fine powder. Decoction was created by extracting the raw sample (20 gm) with methanol (70 ml) by using Soxhlet extraction equipment. The decoction was poised after 5 cycles and will be used for the following estimation [6].

Phytochemical analysis

Phytochemical analysis of MECM was carried out in accordance with standard procedures for identifying flavonoids, alkaloids, tannins, saponins, sterols, and terpenoids [7].

Treatments

Castor oil induced diarrheal method

For 2 days, Group 1(control) received 2ml/kg i.p saline. Group 2 and 3 (test) received 100mg/kg and 200mg/kg methanolic extract of *Citrus maxima*. Before 1hour castor oil (inducing agent) administration was done. We counted both wet and dry diarrheal droppings every 4 hours for Group 4 (standard), who received loperamide (5 mg/kg) as standard [8].

***In Vitro* carminative activity by acid-base titration technique**

The decoctions constituted 5 and 10 milliliters and were placed into conical flasks, and 100 milliliters of distilled water were added. Approximately 100ml of NaOH solution [1M, formerly standardized with oxalic acid] were poured into a plastic container. Citrus maxima are suspended within a reaction vessel that is equipped with an aeration system. After stirring the flask manually for 45 minutes and placing it in a room through the night, the flask will begin to evolve. A container for collecting CO_2 gas discharged from the reaction vessel was let into the reaction vessel. Excess NaOH has been absorbed in the form of Na_2Co_3 (sodium carbonate) and converted to an equivalent quantity. A mixture containing an abundance of sodium hydroxide (NaOH) and sodium carbonate (Na2CO3), salts of which were titrated with standard HCl by using phenolphthalein as an indicator. First, we need to dope out the first end point, and then simultaneously it is important to riddle out the second end point using methyl orange as the indicator.

To estimate the concentration of carbon dioxide (CO_2) per gram of sample, we used the variation in milliliters between the first and second end points:

Vol. of titrant x molarity of std. acid x mol. Wt. of CO_2 = mass of CO_2 in gm [9].

Castor oil induced diarrheal method

For the test, nutrition was denied the rats for 24 hours, and they had free access to water. They were divided into five groups, with one animal in each group. Diarrhoea was induced by giving 1 ml of castor oil orally.

Group I treated as control (2 ml/kg, ip saline), group II and III treated with extract (100 and 200mg/kg ip), after 1hr castor oil was administered and group IV treated with loperamide (5 mg/kg, standard drug). Fecal consistency, both wet and dry diarrhoeal droppings were counted every four hours. The percentage protection was calculated.

Statistical analysis

The data were demonstrated as mean ± standard error mean (S.E.M). Using one-way and multiple-way analysis of variances (ANOVA), we estimated the importance of variation among the groups. Tests followed by Dunnet's test were regarded as significant if the P-values were less than 0.05.

RESULT

Tests of carminative activity using pH-Acid Base titration

Table 1: *In Vitro* carminative activity of methanolic extract of *Citrus maxima*.

Volume of Sample	Difference in Titration value (mL)	Mass of CO_2 (gm)
5mL	1.3mL	4.74
10mL	2.2mL	8.02

Table1

When titrated, 5ml of sample evolved 4.74grams of CO_2 and 10ml of sample evolved 8.02grams of CO_2. According to the results, the most carminative activity is found in 10ml sample. The values are the mean of three titration values.

Castor oil induced diarrheal method

Table 2: Effect of methanolic extract of *Citrus maxima* on castor oil induced diarrhea.

Experimental groups	Dose(mg/kg)	No. of mice with diarrhoeal dropping within 4hrs	Percentage protection (%)
Control	1ml	6	0
Extract-1	100mg/kg	5	16.6
Extract-2	200mg/kg	3	50
Loperamide (standard)	5mg/kg	0	100

Table 2

The percentage (%) protection of extract 2- 50%, which is higher than the percentage (%) protection of extract 1- 16.6%. In comparison with extract 1, extract 2 has a higher anti diarrheal effect.

DISCUSSION

***In vitro* carminative activity by acid-base titration technique**

Frequently, flatulence (formation of gas) occurs when carbohydrates are not properly digested. Because flatulence is controlled by antacid, the adverse effect is homogenous with that of acid reflux. Results of the current study proved that the MECM has carminative properties. Aside from the fact that the drug extract has a strong carminative effect, it also releases a high volume of carbon dioxide compared to regular sodium bicarbonate. Methanolic extract of *C. maxima* contains

a high amount of fibers and other nutrients which may enhance carminative activity. Therefore, it is inferred from the study that the *C. maxima* leaf has the potential to decrease acidity & flatulence. From Table 1, Dose dependent carminative activity is observed with the extract.

Castor oil induced diarrheal method

In this study MECM has been shown to reduce castor oil induced diarrheal episodes, intestinal secretion and motility. Hence, these results can be regarded as scientific evidence that this plant has antidiarrheal properties. Moreover, the *In vivo* Anti Diarrheal index (ADI) that measures the delay of diarrhea, inhibition of wet stool output, and intestinal transit in the treated rats when compared to a negative control group indicates the dose dependent action of the plant. However, it has not been determined exactly how the plant extract produces these effects. It is possible to propose a mechanism of action for the extract depending on the pathophysiology of diarrhoea and the action of castor oil in inducing diarrhoea.

CONCLUSION

The present study establishes carminative and antidiarrheal properties. Reduction in flatulence effect by production of large amounts of CO_2 shows potent carminative effect. Inhibition of defecation frequency and wetness of fecal excretion similar to loperamide proved antidiarrheal activity. Further studies are required to monitor antidiarrheal activity in various models of diarrhea and examination of active principles.

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