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RESEARCH ARTICLE

ANTIDIARRHOEAL AND ANTIMICROBIAL ACTIVITIES OF THE METHANOLIC EXTRACT OF THE FLOWERING TOPS OF *ROSA DAMASCENA*

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ABSTRACT

The present study was carried out to investigate possible antidiarrhoeal and antibacterial activities of methanolic extract of the flowering tops of *Rosa damascena*. Antidiarrhoeal activity of the methanolic extract of the flowering tops of *Rosa damascena* was tested by castor oil induced diarrhoea in mice, it increases mean latent period and decreased the frequency of defecation significantly and dose dependently at the doses of 250 mg/kg ($P < 0.005$) and 500 mg/kg ($P < 0.001$) body weight comparable to the standard drug loperamide at the doses of 50 mg/kg body weight. The antimicrobial activity was investigated against two Gram positive and nine Gram negative bacteria by using disc diffusion method. The methanolic extract showed moderate antimicrobial activity against Gram positive bacteria namely *Staphylococcus aureus,* and Gram negative bacteria namely *Escherichia coli, Shigella boydii, Shigella flexneri, Enterococci spp,* and *Samonella typhi* in dose dependant manner. The present study clearly supports the medicinal value of this plant. The overall results indicate the possibility of presence of some active principles in the plant extract possessing antidiarrhoeal and antibacterial actions.

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INTRODUCTION

Medicinal plants have been used for the treatment of diseases since the early civilizations of the Middle East, India, China and the New World (Ferrence and Bendersky, 2004). Over centuries and decades our ancestors relied on the herbal products as therapeutic which can be traced back to at least 5,000 years (Rajendran, 2009). According to World Health Organization about 80% of the world population depends on the natural product for their health due to minimal side effect and cost effective (Jagtap *et al*., 2009). *Rosa damascena* Mill. (Rosaceae) is a well known shrub. Members of the genus Rosa are considered as one of the world's most popular ornamental plants because of their beauty and fragrance (Das *et al*., 2012). Some people also called it as Gule Mohammadi "flower of Prophet Mohammad" because they believe pleasant aroma of the flower reminds them of Prophet Mohammad (Nikbakht, 2008).

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Rosa damascena is cultivated in rose gardens in several places in Bangle, Kashmir and Punjab. Enormous quantity of wild hill roses grows throughout the North West Himalayas and Kashmir (Joshi, 2004). It is an important medicinal plant of ancient system of medicine which process different pharmacological action such as antioxidant, hepatoprotective, CNS depressant, anti-inflammatory, anti HIV, Laxative, Cardio and Brain tonic (IBN, 2007; Abdul Hakeem, 2002; Ghani, 2010; Abdul Hakeem, ?; Kabeeruddeen, 2007; Nyeem *et al*., 2006). Essential oil, quarcetin, kampferol and cyanidin have been isolated from whole plant (Joshi, 2004). Cyanidine 3, 5-diglucoside has been isolated from petals. Flowers also contain a bitter principle, tanning matter, fatty oil and organic acids (Joshi, 2004). The tetrahydroxyflavanone (kaempferol) isolated from the methanol extract was effective in reducing the maturation of infectious progeny virus apparently due to selective inhibition of the viral protease (Mahmood, 1996). On the other hand the pentahydroxyflavone (quercetin) and two 3 substituted derivatives of kaempferol appeared to inhibit HIV-infection by preventing binding of gp120 to CD4 and 2-Phenylethanol-O-(6-O-galloyl)-beta-D-glucopyranoside

interacted irreversibly with gp120 and neutralized virus infectivity (Mahmood, 1996). The essential oil of *Rosa damascena* petals was evaluated for its antibacterial effects against three strains of *Xanthomonas axonopodis* spp. Vesicatoria (Basim, 2003). The rose teas were rich in free gallic acid. The highest values of antioxidant activity, total phenols and gallic acid contents were found (Vinokur *et al*., 2006). Achuthan *et al*. reported that *Rosa damascena* could protect against CCl4 induced hepatotoxicity, possibly by its free radical scavenging activity (Achuthan *et al*., 2003). *Rosa damascena* also has a high ability to inhibit lipid peroxidation in rat model (Shahriari *et al*., 2006).

In developing countries, a majority of people living in rural areas almost exclusively use traditional medicine in treating all sorts of diseases including diarrhoea. Diarrhoea is a major health problem especially for children under the age of 5 and up to 17% of children admitted in the paediatrics ward die of diarrhoea. Worldwide distribution of diarrhoea accounts for more than 5 million deaths each year in infants and children below 5 years and old especially in developing countries (Snyder and Merson, 1982; Park, 2000). The incidence of diarrhoeal diseases still remains high despite the efforts of many governments and international organizations to control it. It is therefore important to identify and evaluate available natural drugs as alternatives to currently used anti-diarrhoeal drugs, which are not always free from adverse effects (Hardman and Limberd, 1992). A range of medicinal plants with antidiarrhoeal properties is widely used by traditional healers. However, the effectiveness of many of these antidiarrhoeal traditional medicines has not been scientifically evaluated.

A survey of the published literature shows that there are a number of different methods used for the assessment of antimicrobial activity; however, there is no one method that is used by all researchers and no inclusive study to determine which one is the best method for in vitro assay (Wilkinson, 2007). Majority of the researchers uses one of the three following methods for the assessment of antimicrobial activity: Disc diffusion, agar dilution, and broth dilution/microdilution method. The disc diffusion method (also known the zone of inhibition method) (Barry, 1980; Bauer, 1966; Rahman and Rashid, 2008) is probably the most widely used of all methods used for testing antibacterial and antifungal activity (Wilkinson, 2007). It requires only small amounts of the test substance (10-30 ml), can be completed by research staff with minimal training and as such may be useful in field situations (Wilkinson, 2007). Several researchers have used the method to identify the antibacterial and antifungal activities of the plant extracts (Belboukhari and Cheriti, 2006), compounds isolated from plants (Khan *et al*., 2008) and also to find out the antimicrobial resistant strains of microorganisms (Hallander and Laurell,1972; Vedel, 2005). One of the major problems that concerns public health, is bacterial resistance against antibiotics (Karlowsky *et al*., 2012; Gayathri *et al*., 2013). Therefore, researchers have been screening natural sources for as yet undiscovered antimicrobial agents (Srivastava *et al*., 1996; Ahmed *et al*., 2013; Mahesh and Satish, 2008).

The aim of this study was, therefore, to evaluate the antidiarrhoeal activities of methanolic extracts of the flowering tops of *Rosa damascena* in experimentally induced diarrhoea in rats and antimicrobial activities of the same extracts over some enterobacteria strains.

MATERIALS AND METHODS

Plant material

The flowering tops of *Rosa damascena* were collected from the Gazipur, Dhaka, Bangladesh and identified by the experts of Bangladesh National Herbarium, Dhaka and the sample has been preserved in Pharmacology Laboratory, Bangladesh Agricultural University.

Preparation of methanol extracts

The flowering tops of *Rosa damascena* was washed under tap water and were efficiently dried under shade for about one week and protect from deterioration. The shade dried flower was grinded made into powder with the help of laboratory mixer. When the moisture content was found to be below 5% by loss on drying (LOD) at 100˚c of the dried flowering tops. The flowering tops were powdered and weighed (400gm) and then extracted with 95% of methanol in a Soxhlet apparatus at an elevated temperature. The extract was concentrated by evaporation under reduced pressure at 40°C using Buchi rotary evaporator to yield a gummy reddish black colored extract (yield appx. 5.7%).

Animals

Male and female Swiss mice of either sex (20-25 g body weight) bred in the animal house of the Department of Pharmacology, Bangladesh Agricultural University, were collected from the animal resources branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B).The animals were housed under standard laboratory conditions (relative humidity 55-60%, r.t 23±2^{o}c and 12 h light: dark cycle).The animals were fed with standard diet and water *ab libitum.* The animals were divided in groups of 4, with each group balance for sex and body weight. The institutional animal ethical committee approved the study protocol.

Acute Toxicity Test

Acute oral toxicity (Ecobichon, 1997) study was performed as per OMCD-423 guidelines. Test animals (n = 6) of either sex selected by random sampling technique were for the study. The animals were kept fasting for overnight providing only water, after which the methanolic extracts were administrated orally at the dose label of 5 mg/kg body weight by intragastric tube and observed for 14 days .If mortality was observed in 2-3 animals, then the dose administrated was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 50,300, and 2000 mg/kg body weight

Drug

Loperamide (Square Pharmaceuticals Limited, Bangladesh)

Pharmacological Studies

Antidiarrhoeal activity

Antidiarrhoeal activity of the methanolic extract of the flowering tops of *Rosa damascena* was tested using the model castor oil induced diarrhoea in mice (Racusen and Binder, 1979). The mice were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the final experiment. The test animals were randomly chosen and divided into three groups having six mice in each. Group-I was kept as control and received vihicles only (distilled water containing 0.1% Tween-80); group II was treated as 'positive control' and was given the standard drug loperamide at dose of 50 mg/kg of body weight; group III and group IV (test group) were treated with the extract of *Rosa damascena* at dose of 250 mg/kg body weight and 500 mg/kg of body weight. Control vehicle, standard drug and the extract were administered orally.

After one hour treatment with distilled water, standard drug or plant extract, each animal was given 0.5 ml of castor oil by oral route. Individual animals of each group were then placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhoea every hour in five hours study after the castor oil administration. Number of stools or any fluid material that stained the adsorbent paper were counted at each successive hour during the 4-hour period and were noted for each mouse. The latent period of each mouse were also counted. At the beginning of each hour new papers were placed for the old ones.

Antimicrobial activity

The antimicrobial assay was performed by disc diffusion method (Barry, 1980; Bauer, 1966; Rahman and Rashid, 2008). Eleven pathogenic bacteria (collected from the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B) were inoculated on 16 ml previously sterilized nutrient agar media, mixed thoroughly and transferred immediately to the sterile Petri dish in an aseptic condition using a sterile loop.

Prepared plant extracts (250µg/disc and 500µg/disc) and standard antibiotic ciprofloxacin solutions (5µg/disc) were applied to the corresponding Petri dish. The plates were incubated for 24 hours at 37˚C. After proper incubation, clear zone of inhibition around the point of application of sample were measured and expressed in millimeter (mm).

RESULTS AND DISCUSSION

Antidiarrhoeal activity

Antidiarrhoeal activity of the methanolic extract of the flowering tops of *Rosa damascena* was tested by castor oil induced diarrhoea in mice (Racusen and Binder, 1979). The extract caused an increase in latent period (1.16 ±0.09 ($P < 0.005$) and 2.1 ±0.12 ($P < 0.001$) hour) i.e. delayed the onset of diarrhoeal episode at the dose of 250 mg/kg and 500 mg/kg body weight which was comparable to the standard drug loperamide at the dose of 50 mg/kg body weight in which the value was 2.58 h (Table 1). The extract also significantly decreased the frequency of defecation at the same dose where the mean numbers of stool at the 1st, 2nd, 3rd and 4th h of study were 7 hours and 5.8 hours respectively and in standard drug the values were 3.6 hours respectively (Table 1). Castor oil produces diarrhoea due to its most active component recinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion (Gaginella *et al*., 1975). The methanolic extract of the flowering tops of *Rosa damascena* significantly and dose dependently decreased the onset of diarrhea in mice at he tested doses. The maximum effect was found at 500 mg/kg of body weight.

Table 1. Effect of the methanolic extract of flowering tops of *Rosa damascena* on castor oil induced diarrhoea in mice

Treatment	Total number of stools in 4 hour	Mean of stools in 4 hour	Mean of latent period (hour)
Control (Tween 80)	45	9	0.64 ±0.05
Positive control (Loperamide 50 mg/kg)	18	3.6	2.58 ±0.13
Test Group -1 (250 mg/kg body weight)	35	7	1.16 ±0.09[b]
Test Group -1 (500 mg/kg body weight)	29	5.8	2.1 ±0.12[a]

[a]p<0.001 [b]p<0.005 vs. control, Values are expressed as mean ± SEM (n = 6)

Bacterial strains	Diameter of zone of inhibition in millimeter (mm)		
	Ethanol Extract (250 µg/disc)	Ethanol Extract (500 µg/disc)	Ciprofloxacin (5 µg/disc)
Gram negative			
Escherichia coli	6	7	31
Vibrio cholera	6	6	27
Shigella dysenteriae	6	6	28
Shigella boydii	6	9	30
Shigella flexneri	6	10	29
Shigella sonnei	6	6	25
Enterococci spp	6	8	28
Pseudomonas spp	6	6	30
Samonella typhi	6	9	31
Gram positive			
Staphylococcus aureus	6	10	27
Staphylococcus epidermis	6	6	30

On the basis of the result of castor oil induced diarrhoea, it can be concluded that the methanolic extract of the flowering tops of *Rosa damascena* might possess a significant antidiarrhoeal activity.

Antimicrobial activity

Antimicrobial activity of the flowering tops of *Rosa damascena* was tested by using the disc diffusion method Barry, 1980; Bauer, 1966; Rahman and Rashid, 2008). The antimicrobial activity possess against 09 gram negative and 02 gram positive test organisms at the doses of 250 µg/disc and 500 µg/disc and the results were compared with the standard antibiotic ciprofloxacin (5 µg/disc). Among the test organisms the extract showed moderate dose dependent antibacterial activity against *Escherichia coli, Staphylococcus aureus, Shigella boydii, Shigella flexneri, Enterococci spp,* and *Samonella typhi* and no activity against *Shigella sonnei, Shigella dysenteriae, Pseudomonas spp, Staphylococcus epidermis.* The result of the antimicrobial activity, measured in term of diameter of zone of inhibition in millimeter (mm). Antimicrobial activity of the plant extract against both Gram posi- tive and Gram negative bacteria may be due to the presence of broad spectrum antibiotic compounds (Cichewicz and Thorpe,1996; Srinivasan *et al*., 2001) or the previously reported compounds like essential oil, rich in methyl chavicol (Garg, 2001).

Conclusion

The results of the present study indicate that the methanol extracts of the flowering tops of *Rosa damascena* possess significant antidiarrhoeal and antmicrobial potentials in dose dependant manner. The present data justify the traditional uses of this plant for the treatment of various diseases. However, further studies are required for isolation and purification of the active principles of the plant responsible for these effects and to better understand the mechanism of such actions.

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