Asian Journal of Chemistry; Vol. 23, No. 4 (2011), 1767-1770

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Antidiarrhoeal Activity of Methanolic Extract of *Acacia farnesiana* Bark

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(*Received*: 29 July 2010; *Accepted*: 22 December 2010) AJC-9409

Based on ethno-botanical use the bark of *Acacia farnesiana* Willd. (family-Mimosaceae) was studied for the antidiarrhoeal activity. Bark of *Acacia farnesiana* was successively extracted with petroleum ether, methanol and distilled water. The extracts were dried under reduced temperature and pressure. The methanolic extract of *Acacia farnesiana* bark (AFM) showed significant antidiarrhoeal activity in exploratory studies. The methanolic extract of *Acacia farnesiana* bark at 100, 200 and 400 mg/kg, p.o. produced a significant and dose dependent inhibition of in castor oil induced diarrhoea. Therefore methanolic extract of *Acacia farnesiana* bark was studies in detail for magnesium sulphate induced diarrhoea, intraluminal fluid accumulation and normal as well as barium chloride induced charcoal transit. Antimicrobial activity of methanolic extract of *Acacia farnesiana* bark against diarrhoea causing micro-organisms has also been studied *in vitro*. The methanolic extract of *Acacia farnesiana* bark showed significant inhibition of castor oil and magnesium sulphate induced diarrhoea. It also inhibits fluid accumulation against castor oil challenge in enteropooling assay. The methanolic extract of *Acacia farnesiana* bark reduced normal as well as barium chloride induced peristalsis of small intestine in mice. The methanolic extract of *Acacia farnesiana* bark also showed antimicrobial activity against common pathogens responsible for diarrhoea *in vitro*.

Key Words: *Acacia farnesiana* Willd., Antidiarrhoeal activity, Antimicrobial activity, Castor oil charcoal meal, Swiss albino mice.

INTRODUCTION

Acacia farnesiana Willd. (family-Mimosaceae) is a thorny shrub commonly found in India. Pods are rich source of tannins. Leaves are used for culinary purposes. Wood is used of building of ships[1]. Roots are used in the treatment of snakebite, leaves are used for the treatment of gonorrhoea, bark is astringent and used for the treatment of inflammation, diarrhoea, dysentery, bronchitis and cough[2]. Earlier *Acacia farneisana* has been explored scientifically for bronchodilator, antiinflammatory[3] antimalarial[4] abortifacient and diuretic activity[5]. Flavonoids vicenin-2, linamarin has been isolated from the aerial parts of *Acacia farnesiana*[6,7].

In developing countries diarrhoea infectious as well as non-infectious is a major health care problem and is a leading cause of mortality and morbidity especially among children[8]. Many microbial species like *Escherichia*, *Staphylococcus*, *Shigella* and *Salmonella* are causative agent for diarrhoea[9]. Traditionally medicinal plants used for the treatment of various disesese including diarrhoea constitute an indispensable because of economical viability, accessibility and ancestral experience. Vast spectrum of approaches for management of diarrhoea are available, still majority of the people of the developing countries rely on herbal drugs for the management of diarrhoea. World Health Organization (WHO) has encouraged studies for treatment and prevention of diarrhoea depending on traditional medical practices[10]. The ethno-botanical use of bark of *A. farnesiana* for the treatment of inflammation, diarrhoea, dysentery is recorded but such practices are followed empirically therefore it is very difficult to predict exact utility as well as limitations of such treatments. Till date there is no scientific data is available on antidiarrhoeal effects of *Acacia farnesiana* bark, therefore attempt has made to verify the claimed biological activities of this plant for possible antidiarrhoeal (*in vivo*) and antimicrobial (*in vitro*) properties.

EXPERIMENTAL

Acacia farnesiana bark was collected from Pune and its surrounding areas during May-June 2007. The herbarium specimen (AHMA: 24396) was deposited in Agharkar Herbarium of Maharashtra Association at Agharkar Research Institute, Pune (ARI), Pune, India.

Activated charcoal, barium chloride, magnesium sulphate, E.-Merck (India) Limited, Castor oil (refined pure)-Paras Chemical Industries, Pune, chlorpromazine hydrochloride-Rhone Poulene (India) Limited, diphenoxylate hydrochloride-Searle, India, loperamide hydrochloride-Cipla pharmaceuticals Ltd., Mueller Hinton agar and Octodiscs-nalidixic acid 30 mg Hi Media-Solvents-SQ grade of Qualigens fine chemicals.

Swiss albino mice of either sex, weighing between 20-25 g were used for all the experiments. Earlier they were obtained from the National Institute of Virology, Pune, India and have been inbred in the animal house facility at Agharkar Research Institute for several generations for the last 25 years. They are housed in an air-conditioned area at 25 ± 2 ºC, in polypropylene cages with 10:14 h light and dark cycle. Amrut brand balanced animal feed and water *ad libitum* were given. For pilot experiments three and for final experiments group of six mice was used for each treatment. Protocols of animal studies were approved by Institutional Animal Ethics Committee (IAEC) of Agharkar Research Institute.

Microorganisms: All microbial cultures were obtained from Microbial Sciences Division of ARI.

Staphylococcus aureus MCMB-818, *Escherichia coli* MCMB-813, *Pseudomonas aeruginosa* MCMB-816, *Bacillus cereus* MCMB-817, *Shigella sonnii* MCMB-815, *Enterobacter fecalis* MCMB-812. Microbial cultures were grown on Mueller-Hinton agar at 37 ºC for 12-14 h. They were maintained at 4 ºC in the laboratory.

Extraction of plant material: *A. farnesiana* bark was cleaned properly to remove soil and dirt. The bark was shade dried and then coarsely powdered. The powder was successively extracted with petroleum ether (60-80 ºC), methanol and distilled water by cold maceration method. These extracts were concentrated at reduced temperature and pressure using rotary evaporator and completely freed of solvents. Yields of petroleum ether, methanol and water extracts were 0.94 and 4.38 and 2.54 % w/w, respectively.

Standardization of plant extract: As per the standard procedure the bioactive extract methanolic extract of *Acacia farnesiana* bark was standardized by recording high performance thin layer chromatography (HPTLC) profile. For this study pre-coated aluminum non-fluorescent plates (E. Merck) were used. Linomat IV was used for spotting and all plates were developed using chloroform:methanol:formic acid (80:20:10) solvent system. The chromatograms were scanned at 254 and 366 nm using densitometer-TLC Scanner III, employing "CATS" software (Camag, Switzerland). Three major spots at 254 nm at 0.11, 0.13 and four major spots at 366 nm at R_f 0.36, 0.42, 0.48 and 0.56 were recorded, respectively.

Acute oral toxicity studies: As per revised OECD guidelines No. 423, all the extracts were studied as for acute oral toxicity using limit test protocol. The extracts did not showed any toxicity up to 2000 mg/kg in albino mice by oral route.

Exploratory studies of antidiarrhoeal activity: For initial antidiarrhoeal studies mice were fasted overnight and were distributed into defined groups for the petroleum ether, methanol and water extract treatments (400 mg/kg p.o.). Each animal was placed in a separate cage and number of defecations of each animal was recorded up to 4 h. No significant difference in fecal output was observed in normal and extract treated animals. The same study was repeated after treating animals with 0.1 mL castor oil by oral route. Methanolic extract of *Acacia farnesiana* bark showed significant antidiarrhoeal activity at 400 mg/kg dose; hence for further studies 100-400 mg/kg doses of by oral route.

Castor oil induced diarrhoea: As shown in Table-1, a group of mice were divided into different groups for the treatment of different doses of methanolic extract of *Acacia farnesiana* bark, vehicle or standard diphenoxylate HCl (5 mg/kg; p.o.). 0.1 mL of castor oil was administered by oral route after 0.5 h of respective treatment to each animal. The number of defecations up to 4 h recorded[11].

Magnesium sulphate induced diarrhoea: As indicated in Table-1, mice were divided into different groups for the treatment of various doses of methanolic extract of *Acacia farnesiana* bark, vehicle or standard diphenoxylate HCl (5 mg/kg; p.o.). After 0.5 h each animal was given magnesium sulphate 2 g/kg by oral route. The number of defecations per animal was recorded up to 6 h[12].

Effect on small intestinal secretions: Intestinal secretion of methanolic extract of *Acacia farnesiana* bark was indirectly studied by enteropooling assay. For this study, mice were divided into different groups for various treatments with methanolic extract of *Acacia farnesiana* bark, vehicle and standard chlorpromazine 30 mg/kg i.p. 0.5 h before the oral administration of castor oil 0.2 mL per mouse. These mice were sacrificed after 0.5 h and entire small intestine was dissected out and its weight was determined. The difference in the weight of small intestine in control and castor oil treated group was considered as the castor oil induced fluid secretion in small intestine[13].

Effect on small intestinal transit: Small intestinal transit effect of methanolic extract of *Acacia farnesiana* bark was studied in different groups of mice in normal and barium chloride treated animals as follows: (i) 0.5 h after treatment of

TABLE-1
EFFECT OF METHANOLIC EXTRACT OF *Acacia farnesiana* BARK ON CASTOR OIL AND MAGNESIUM SULPHATE INDUCED DIARRHOEA IN ALBINO MICE

Treatment	No. of defections at various hours, mean ± SEM					
	1	2	3	4	5	6
Castor oil control	10.90 ± 0.80	17.00 ± 1.46	20.10 ± 1.32	24.3 ± 0.90	–	–
AFM (100 mg/kg)	6.00 ± 0.52	7.33 ± 0.4*	9.00 ± 0.52*	9.83 ± 0.48*	–	–
AFM (200 mg/kg)	3.83 ± 0.66*	6.67 ± 0.86*	8.67 ± 0.77*	10.50 ± 2.33*	–	–
AFM (400 mg/kg)	4.33 ± 0.57*	6.50 ± 0.57*	8.83 ± 0.80*	10.33 ± 0.62*	–	–
Dipheynoxylate HCl (5 mg/kg)	2.00 ± 0.22*	3.33 ± 0.83*	9.83 ± 0.66*	11.67 ± 0.21*	–	–
Magnesium sulphtate control	8.43 ± 1.51	17.57 ± 1.78	20.43 ± 1.58	21.86 ± 1.52	27.57 ± 1.95	32.14 ± 2.75
AFM (100 mg/kg)	7.17 ± 0.71*	9.67 ± 0.72*	15.83 ± 1.15*	17.67 ± 0.62*	20.00 ± 1.58*	23.67 ± 0.93
AFM (200 mg/kg)	4.67 ± 0.56*	12.50 ± 1.43*	14.50 ± 0.71	14.83 ± 0.71*	17.17 ± 1.71*	18.17 ± 0.61*
AFM (400 mg/kg)	3.50 ± 0.43 *	12.50 ± 0.43*	14.33 ± 0.56*	15.50 ± 0.86*	16.33 ± 0.43*	17.00 ± 0.52*
Diphenoxaylate HCl (5 mg/kg)	4.67 ± 0.56*	11.83 ± 0.48*	14.00 ± 0.71*	14.83 ± 0.71*	17.17 ± 0.61*	18.20 ± 0.76*

N = 6, *Significant as compared to respective control $p < 0.05$; AFM = Methanolic extract of *Acacia farnesiana* bark.

methanolic extract of *Acacia farnesiana* bark, vehicle or standard mice were administered with 0.2 mL of charcoal meal (3 % charcoal in 5 % gum acacia) by oral route. After 20 min all animals were sacrificed and distance traveled by charcoal with reference to total length of small intestine was calculated for each mouse[13]. The percentage of distance traveled is shown in Table-3. (ii) In second experiment, after 20 min treatment of methanolic extract of *Acacia farnesiana* bark or vehicle or standard drug loperamide hydrochloride, mice were administered with barium chloride 5 mg/kg by i.p. route. Activated charcoal 0.2 mL (3 % charcoal in 5 % gum acacia) was administered to each mouse by oral route after 10 min. After 0.5 h each mouse was sacrificed and distance traveled by charcoal with respect to the total length of intestine was measured to express percentage of distance traveled in each group[14] as shown in Table-3.

Antimicrobial activity: Antimicrobial activity of methanolic extract of *Acacia farnesiana* bark was studied using modified agar well diffusion technique. Each microbial culture of an 18 h age was diluted in sterile saline to get an absorbance of 0.11 on UV spectrophotometer at 620 nm. Using sterile glass spreader 0.1 mL of culture was inoculated on plates containing Mueller-Hinton agar and spread evenly. All the plates were incubated at 37 ºC for 0.5 h. 5 mm wells were made by borer and different concentrations of extracts were added along with control (DMSO) and octo-discs of nalidixic acid 30 mg was used as positive control. The plates were incubated at 37 ºC for 18 h and zones of inhibition were recorded in millimeters and the mean values were calculated to study antimicrobial activity[15].

Statistical analysis: The results of all experiments were reported as mean ± SEM. The results were further analyzed by using Student's t-test and significance of the results are expressed in terms of p values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Effect of methanolic extract of *Acacia farnesiana* bark on castor oil induced diarrhoea: Methanolic extract of *Acacia farnesiana* bark showed dose dependent inhibition of castor oil induced diarrhoea in albino mice (Table-1). This effect was significant at 200 and 400 mg/kg doses of methanolic extract of *Acacia farnesiana* bark at 4 h when compared to control group; but the activity was less potent when comparable with diphenoxylate HCl.

Effect of methanolic extract of *Acacia farnesiana* bark on magnesium sulphate induced diarrhoea: As shown in Table-1, methanolic extract of *Acacia farnesiana* bark is inhibiting magnesium sulphate induced diarrhoea in dose dependant way in albino mice. This effect was significant at higher doses of methanolic extract of *Acacia farnesiana* bark up to 6 h when compared to control group; this activity was comparable with standard drug.

Effect of methanolic extract of *Acacia farnesiana* bark on small intestinal secretion: The castor oil induced intraluminal fluid accumulation was significantly inhibited by methanolic extract of *Acacia farnesiana* bark in a dose dependant manner. The results are well comparable with standard drug at higher dose of methanolic extract of *Acacia farnesiana* bark. The results are shown in Table-2.

TABLE-2
EFFECT OF METHANOLIC EXTRACT OF *Acacia farnesiana* BARK (AFM) ON CASTOR OIL INDUCED INTRALUMINAL FLUID ACCUMULATION IN ALBINO MICE

Treatment	Weight of small intestine/20 g of mice, mean ± SEM	Castor oil induced intraluminal fluid (mg)
Control	0.865 ± 0.095 g	–
Castor oil	1.709 ± 0.046[a] g	844
AFM (100 mg/kg)	1.830 ± 0.093 g	965
AFM (200 mg/kg)	1.473 ± 0.075 g	608
AFM (400 mg/kg)	0.997 ± 0.177* g	132
Chlorpromazine HCl (30 mg/kg)	1.115 ± 0.0217[b] g	250

N = 6 in each group, *Significant as compared to castor oil group $p < 0.05$, [a]Significant as compared to control group $p < 0.05$, [b]Significant as compared to castor oil group $p < 0.05$

Effect of methanolic extract of *Acacia farnesiana* bark on small intestinal transit: methanolic extract of *Acacia farnesiana* bark is significantly inhibiting the normal gastrointestinal transit of charcoal in mice at 200 and 400 mg/kg doses. The result of standard drug loperamide is very well comparable with extract dose 400 mg/kg. Barium chloride induced gastrointestinal transit of charcoal is also significantly inhibited in mice only at 400 mg/kg of methanolic extract of *Acacia farnesiana* bark. The results are shown in Table-3.

TABLE-3
EFFECT OF METHANOLIC EXTRACT OF *Acacia farnesiana* BARK (AFM) ON GASTROINTESTINAL TRANSIT IN MICE

Treatment	Distance traveled by charcoal (as percentage of total length of small intestine) mean ± SEM	Inhibition (%)
Normal control	67.65 ± 5.00	–
AFM (100 mg/kg)	59.47 ± 2.12	12.10
AFM (200 mg/kg)	58.70 ± 1.61	13.23
AFM (400 mg/kg)	51.79 ± 1.49*	23.35
Loperamide (5 mg/kg)	38.04 ± 2.73*	43.77
$BaCl_2$ control	75.37 ± 1.32*	–
AFM (100 mg/kg)	71.77 ± 4.24	4.78
AFM (200 mg/kg)	68.74 ± 1.23[a]	8.80
AFM (400 mg/kg)	50.80 ± 3.07[a]	35.30
Loperamide (5 mg/kg)	48.56 ± 2.97[a]	35.38

*Significant as compared to normal control $p < 0.05$. [a]Significant as compared to barium chloride control $p < 0.05$.

Antimicrobial activity of methanolic extract of *Acacia farnesiana* bark: Methanolic extract of *Acacia farnesiana* bark showed significant antimicrobial activity against all microbial strains however, this activity is less as compared with standard nalidixic acid (30 µg) (Fig. 1).

Diarrhoea is the frequent passage of liquid faeces and it involves both an increase in the motility of the gastrointestinal tract along with increased secretion and decreased absorption of fluid which results in loss of electrolytes (particularly sodium) and water[16]. To control symptom of diarrhoea, antibiotics are used however it cannot inhibit the toxicity of toxins liberated by microbial agents. Moreover, antibiotic therapy is

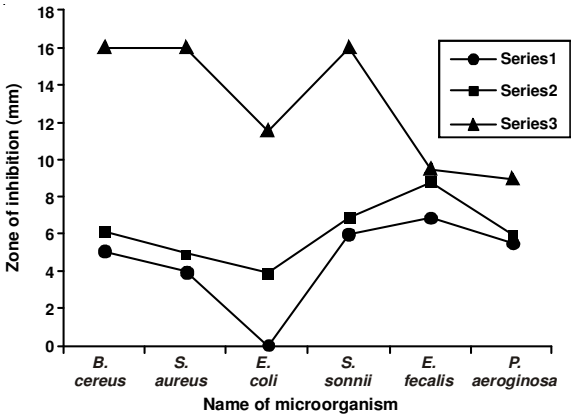

Fig. 1. Antimicrobial activity of AFM. Series 1: AFM 100 µg/well, series 2: AFM 200 µg/well, series 3-standard (nalidixic acid 30 µg)/well

not a viable solution because of the rapid increase in antibiotic resistance, particularly in endemic areas[17]. Several mechanisms have been proposed for the diarrhoeal effect of castor oil[18]. These are inhibition of intestinal Na^+, K^+ ATPase activity to reduce normal fluid absorption, activation of adenylate cyclase or mucosal cAMP-mediated active secretion and stimulate prostaglandin synthesis, platelet-activating factor and recently nitric oxide has been claimed to contribute to the diarrhoeal effect of castor oil[19].

One of the well known mechanisms of castor oil induced diarrhoea is through active metabolite ricinolic acid[20]. Ricinolic acid significantly increases peristaltic activity and also produces permeability changes in the mucosal membrane of intestine to electrolytes and water. The castor oil induced diarrhoea is also reported through increase in prostaglandin biosynthesis[11,21,22]. The increased prostaglandin levels also alter physiological function of gastrointestinal tract leading to diarrhoea[23]. The methanolic extract of *Acacia farnesiana* bark is also showed action against magnesium sulphate induced diarrhoea, which is osmotic in nature. It also increases the volume of the intestinal content through prevention of reabsorption of water. It promotes release of cholecystokinin from duodenal mucosa and consequently increases the secretion and motility of the small intestine therefore prevents the reabsorption of water and electrolyte[22,24].

The extract may increase the re absorption of water and electrolyte from the gastrointestinal mucosa as well as delays the transit of charcoal in normal as well as barium chloride induced increase peristalsis mice as compared to the control. The delay in the gastrointestinal transit time by the extract might have contributed at least up to some extent to their antidiarrhoeal activity of the extract.

Based on ethno medicinal use the studies were carried on various extracts of *A. farnesiana* bark to validate antidiarrhoeal activity. The methanolic extract of *Acacia farnesiana* bark also possesses promising antimicrobial activity against the microbes causing diarrhoea and dysentery. Use of locally available medicinal plants for the treatment of diarrhoea is a common practice in traditional systems as well as in folk medicines. For universal acceptance of such treatments it is necessary to understand probable/precise mechanism of action and limitations. Since the methanolic extract exhibit antidiarrhoeal activity along with antimicrobial activity the used of bark of *A. farnesiana* for antidiarrhoeal activity is justified. Further isolation and identification of active compound responsible for antidiarrhoeal activity is in progress.

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