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Original Research Article

The Antidiarrheal Activity and Phytoconstituents of Some Methanol Extracts from *Asteraceae* family

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Abstract

The purpose of the present study was to evaluate the antidiarrheal activity and the phytochemical constituents of methanol extracts of some *Asteraceae* used traditionally in kingdom of Saudi Arabia using castor oil – induced diarrhea and gastrointestinal motility test using charcoal meal method were examined. The methanolic extracts were initially assayed for their effects in castor oil – induced diarrhea at different doses (250 and 500 mg/kg b.wt. followed by their evaluation on the peristaltic movements in charcoal meal test. The results of the present study indicates that, administration of the following methanol plant extracts; *Achillea fragrantissima* and *Artemisia herba alba* induced a higher percentages of inhibition of diarrhea. While the administration of *Rhanterium epapposum*, *Echinops spinosus* and *Echinops hussoni*, were the least efficacy. The antidiarrheal activity of these plants might be due to their high contents of flavonoids and tannins. It could be concluded that, the remarkable antidiarrheal activity of these plants attest to their utility in a wide range of stats of diarrhea.

Key words: Antidiarrheal, *Asteraceae*, Castor oil, Flavonoids, Phytoconstituents, tannins, rats

INTRODUCTION

The plant kingdom is rich in chemical constituents' of antispasmodics that relieve colicky pain. Intact, most remedies used in conventional medicine include at least one antispasmodic of plant origin. They form a very important part of the treatment of gastrointestinal motility disorders (Sadraei *et al.,* 2003). The antispasmodics are considered useful for relieving or calming colicky pains resulting from spasms of the gut muscles and diarrhea due to hyper motility of the gastrointestinal tract. Among the wide range of plant-derived drugs that have relaxant activities on various smooth muscles, papaverine (*Papaver somniferum*) is the one, which is used in the treatment of colic (Mustafa *et al.,* 1995). Muscarinic antagonists like atropine (*Atropa belladonna*) inhibit the contractions of gastrointestinal tract induced by acetylcholine (Ach) (Broadly and Kelly, 1995). Relatively the less polar solvents like methanol and ethanol are very appropriate in extracting the spasmolytic agents of plants. The ethanol extract of *Capparis cartilaginea* inhibited the submaximal contractions of ileum induced by ACh, histamine or serotonin (Gilani and Aftab, 1994). The crude methanol extracts of *Erythrina sigmoidea* stembark were found to have potent anticholinergic effects by decreasing the tone and spontaneous activity of isolated rat ileum induced by carbachol and acetylcholine (Nkeh *et al.,* 1993). Plant-derived antispasmodics include some tropane alkaloids (atropine, hyoscine or scopolamine, hyoscycamine), opium alkaloids (papaverine, codeine, morphine), flavonoids (luteolin, cirsimartin, quercetin, rutin, apigenin, kaempferol, genkwanin) and essential oils (peppermint, caraway, dill, garlic, chamomile, anise) (Sanchez de Rojas *et al.,* 1994).

Asteraceaeous plants are distributed throughout the world, especially at the Kingdom of Saudi Arabia. These plants typically have hairy and aromatic leaves and flat clusters of small flowers on the top of the stem. The species of *Asteraceae* are a source of many biologically active compounds and due to their bio-active properties plants from *Asteraceae* family are commonly used in treatment of various diseases (Jonathan *et al.,*2014; Magdalena *et al.,* 2012; Chengaiah *et al.,* 2010; Darwish and Aburjai, 2010; Maggi *et al.,* 2009; Hammad and

Abdalla, 2007) and little studies were recorded about the antidiarrheal activity of some of the selected species of the *Asteraceae* family for this experiment. So the present study was designed to explore the antidiarrheal effects and the phytochemical constituents of methanol extracts of five plants from the *Asteraceae* family using castor oil – induced diarrhea and gastrointestinal motility test using charcoal meal method were examined.

MATERIALS AND METHODS

Animals

Wister albino mature male rats (180±20 g) were obtained from the Animal House of the College of Agriculture and Veterinary Medicine, Qassim University, Kingdom of Saudi Arabia and housed at a temperature of 22 - 28 ºC and relative humidity of 50–60%, with artificial light from 5.00 a.m. to 4.00 p.m. Animals had free access to tap water and standard rat chow, used for the study. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH publication no. 85-23, revised 1996). The local ethics committee approved the study.

Preparation of plant material

The plants were collected at the flowering stage, in and around Al-Gouf and Qassim Districts, Kingdom of Saudi Arabia (KSA). The collected plant species were identified and confirmed by Dr. A. Al- Sakeer, Department of Botany, College of Agricultural and Veterinary Medicine, Qassim University, Al Qassim, Kingdom of Saudi Arabia and a voucher specimen was deposited in the Department of Botany, College of Agricultural and Veterinary Medicine, Qassim University, Al Qassim, Kingdom of Saudi Arabia for further reference. Shade dried and powdered plant materials were successively extracted as the following. About 300 gm of the powdered plant were soaked in 3000 ml methanol. It was left for 72 hours , the methanol extract was filtered using Whatman No.1 filter paper and the residue was removed with intermittent shaking till obtain methanolic extract. The extracts were filtered through Whatman No. 1 filter paper and concentrated until obtaining paste under vacuum using the rotary evaporator (Rotavapor R-215, Bṻtchi, Switzerland). The extracts were used for evaluation of the anti-diarrheal activity and phytochemical constituents.

Phytochemical analysis

Total phenolics and total Tannins content

Total phenolic content was determined using Folin-Ciocalteu's reagent (Singleton and Rossi, 1965) with some modifications. Few amount of residue (50 mg) was mixed with 2.5 ml of deionised water followed by 0.25 ml of Folin-Ciocalteu's reagent and allowed to react 6 min. Then 2.5 ml of sodium carbonate 7% was added and allowed to stand for 1 hr, then absorption at 765 nm was measured. Measurements were calibrated to a standard curve of prepared gallic acid solution, and the total phenolic was expressed as mg gallic acid equivalent per g of residue. Total tannin in the extracts was determined by a modification of the Folin-Ciocalteu method using poly vinyl poly pyrrolidone (PVPP) to separate tannin phenols from non-tannin phenols (Osoro *et al.,* 2007). About 100 mg of PVPP was added to 1ml sample extract diluted with 1ml water and left 15 min at 4 ^{o}C. After centrifugation, PVPP forms a precipitate with tannins, and the supernatant has only simple phenols. Simple phenols were determined using the Folin-Ciocalteu reagent as previously mentioned. The difference between total and simple phenol values represents the total tannin content, expressed as mg gallic acid equivalents g residue.

Total flavonoids

The flavonoid content was measured using a colorimetric assay (Zhishen *et al.,* 1999). A known weight of extract residue was dissolved in 1 ml methanol was added to a 10 ml volumetric flask. Distilled water was added to make a volume of 5 ml. At zero time, 0.3 ml of 5% w/v sodium nitrite was added to the flask. After 5 min, 0.6 ml of 10% (w/v) $AlCl_3$ was added and, after 6 min, 2 ml of 1M NaOH were added to the mixture, followed by the addition of 2.1 ml distilled water. Absorbance was read at 510 nm against the blank (water) and flavonoid content was expressed as mg quercetin equivalents/ g residue.

Anti-diarrheal activity

Effect on castor oil-induced diarrhea

The methods of Mukherjee *et al.* (1995) and Rani *et al.* (1999) were applied. Rats of both sexes 150–200 g body weight were used. Seven groups of five animals were used. Groups 1-5, each group was given one of the plant extracts orally in a dose of 250 mg/ kg. Group 6, was given atropine sulfate orally (5 mg /kg) as a reference anti-diarrheal drug. While group 7, was given 2% tween 80 solution and kept as control. One hour after administration of the extracts, all animals received 2 ml of castor oil orally and placed in the standard metabolic cages. The numbers of total and soft fecal pellets were calculated at 1, 2, 3, and 4 h after castor oil administration. The previous experiment was repeated using the following dose of the plant extracts 500 mg/kg b.wt.

Table 1. Quantitative Phytochemical Analysis of the Tested Plants

No	Plant name	Quantitative analysis (mg/g of methanolic residue)			
		Total Phenolics	Tannins	Non tannins phenolics	Total flavonoids
1	*Rhanterium epapposum*	124.26	-------	124.26	30.2
2	*Achillea fragrantissima*	55.57	36.62	18.95	80.4
3	*Artemisia herba alba*	94.19	71.60	22.59	99.7
4	*Echinops spinosus*	66.54	20.28	46.26	44.6
5	*Echinops hussoni*	46.86	22.90	23.96	23.8

Table 2. Effect of some methanol plant extracts on gastrointestinal transit using charcoal meal (marker) in rats

Plant name	Total number of feces	Number of diarrheal feces	% inhibition of diarrhea
Rhanterium epapposum (250 mg/ kg)	19.6 ± 1.20	16.6 ± 1.36	3.33
(500 mg/ kg)	20.0 ± 0.94	15.8 ± 1.11	12.11
Achillea fragrantissima (250 mg/ kg)	17.4 ± 0.51*	13.0 ± 0.44*	27.78
(500 mg/ kg)	15.4 ± 0.59*	11.0 ± 0.83*	38.88
Artemisia herba alba (250 mg/ kg)	16.8 ± 0. 85*	12.4 ± 0.50*	31.11
(500 mg/ kg)	14.2 ± 0.58*	9.4 ± 0.51*	47.77
Echinops spinosus (250 mg/ kg)	20.6 ± 0.92	18.2 ± 0.96	- 1.11
(500 mg/ kg)	18.0 ± 1.37	15.8 ± 1.27	12.22
Echinops hussoni (250 mg/ kg)	21.0 ± 0.70	19.8 ± 0.85	- 10.0
(500 mg/ kg)	19.0 ± 1.41	17.2 ± 1.23	4.44
Atropine sulfate (5 mg/kg)	10.2 ± 0.73*	5.4 ± 0.59*	70.00
Control	20.6 ± 1.50	18.0 ± 1.51	--------

*Significantly different compared to corresponding to control $p \geq 0.05$. Values are expressed as means ± s.e. (n = 5).

Effect on gastrointestinal motility in rats (charcoal meal test)

Rats of both sexes 150–200 g body weight were used. Seven groups of five animals were used. Groups 1-5, each group was given one of the plant extracts orally in a dose of 250 mg/ kg. Group 6, was given atropine sulfate orally (5 mg /kg) as a reference anti-diarrheal drug. While group 7, was given 2% tween 80 solution and kept as control. Five minutes after drug administration, 0.5 mL of a 5% charcoal suspension in 10% aqueous solution of gum acacia powder was administered orally to each animal. The animals were killed 30 min later and the abdomen was opened. The percentage distance of the small intestine (from the pylorus to the caecum) moved by the charcoal meal was calculated (Abdullahi *et al.,* 1994). The previous experiment was repeated using the following dose of the plant extracts 500 mg/kg b.wt. % Inhibition = Mc – Md / Mc x 100. Mc: mean distance travelled by charcoal meal; Md: mean distance travelled by drug or extract.

Statistical analysis

Results were expressed as the mean ± standard error (S.E.M.). All statistical analyses were performed by ANOVA and Duncan's multiple-range test with a value of $p < 0.05$ selected as the cut-off for statistical significance.

RESULTS

Phytochemical investigation

The quantitative phytochemical investigation of the tested plants showed the presence of tannins, flavonoids and phenolics in a different quantities (Table 1). The higher content of tannins and flavonoids were present at the following plant methanolic extracts (*Achillea fragranti*-s*sima* and *Artemisia herba*). However the lower content of tannins and flavonoids were present at the following plant methanolic extracts (*Rhanterium epapposum. Echinops spinosus* and *Echinops hussoni*).

Anti-diarrheal activity. Castor-oil induced diarrhea in rats

As shown from table 2, it was observed that, administration of the methanol extract of the following plants (250 or 500 mg/kg b. wt.); *Achillea fragrantissima* and *Artemisia herba* induced a significant decrease in a total number of feces and number of diarrheal feces and a higher percentage of inhibition of diarrhea. While the administration of *Rhanterium epapposum. Echinops*

Table 3. Effect of some methanol plant extracts on gastrointestinal transit using charcoal meal (marker) in rats

Plant name	Total length of intestine (cm)	Distance travelled by marker (cm)	% intestinal transit	% of inhibition
Rhanterium epapposum (250 mg/ kg)	85.6 ± 3.28	63.2 ± 2.51	73.2 ± 1.1.19	6.56
(500 mg/ kg)	85.4 ± 5.77	68.00 ± 6.09	74.96 ± 5.70	4.31
Achillea fragrantissima (250 mg/ kg)	79.8 ± 3.50	57.0 ± 2.54	71.0 ± 1.00	9.36
(500 mg/ kg)	90.6 ± 8.23	53.80 ± 8.27	59.15 ± 6.62*	24.49
Artemisia herba alba (250 mg/ kg)	82.0± 5.74	61.4 ± 6.20	70.8 ± 4.89	9.62
(500 mg/ kg)	82.2± 5.84	47.40 ± 3.67	57.87 ± 3.38*	26.12
Echinops spinosus (250 mg/ kg)	84.6 ± 2.33	57.4 ± 2.20	67.2 ± 1.39*	14.22
(500 mg/ kg)	90.4 ± 6.86	64.40 ± 8.53	70.08 ± 4.57	10.54
Echinops hussoni (250 mg/ kg)	82.4 ± 6.58	64.8 ± 5.19	79.0 ± 2.60	- 0.008
(500 mg/ kg)	85.2 ± 5.03	65.8 ± 6.99	76.54 ± 4.42	2.29
Atropine sulfate (5 mg/kg)	87.4 ± 4.79	33.80 ± 7.32	39.95 ± 9.25*	49.00
Control	91.8 ± 5.85	72.20 ± 7.07	78.34 ± 5.30	

*Significantly different compared to corresponding to control p ≥ 0.05. Values are expressed as means ± s.e. (n = 5).

spinosus and *Echinops hussoni* insignificantly affect the total number of feces and number of diarrheal feces.

Gastrointestinal transit using charcoal meal (marker) in rats

As shown from table 3, it was observed that *Achillea fragrantissima* and *Artemisia herba* induced a significant decrease in gastrointestinal transit using charcoal meal (marker) in rats (250 or 500 mg/kg b. wt.). While, the administration of *Rhanterium epapposum*, *Echinops spinosus* and *Echinops hussoni*, insignificantly affect the gastrointestinal transit using charcoal meal (marker) in rats.

DISCUSSION

Diarrhea is very common problem in many tropical countries and causes deaths throught the world annually (Li-Li *et al.,* 2000). The use of herbal drugs in the treatment of diarrheal diseases is a common practice in many countries. Several medicinal plants have been reported to be effective against diarrhea. The aqueous extract of *Evodia rutaecarpa* fruit had both anti-transit effect and anti diarrheal effects in mice (Gilani and Aftab,1994). Relatively the less polar solvents like methanol and ethanol are very appropriate in extracting the spasmolytic agents of plants. The ethanol extract of *Capparis cartilaginea* inhibited the submaximal contractions of ileum induced by ACh, histamine or serotonin (Sunilson *et al.,* 2009). *Andrographis paniculata, Asparagus racemosus, Butea monosperma, Cassia auriculata*, and others were used for the same purposes (Ammon *et al.,* 1974). Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the alimentary tract, accompanied by an excess loss of fluid in the feces. In some diarrheas, the secretory components predominate, while other diarrheas characterized by hypermotility. The use of castor oil induces – diarrhea as a model is logical in our study because the autacoids and prostaglandin are involved in the causation of diarrhea (Watson and Gordon, 1962; Galvez *et al.,* 1993). The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa , leading to release of prostaglandin which stimulate motility and secretion (Izzo *et al.,* 1999).

The results of the present study show that, the extracts of the following plants *Artemisia herba alba* and *Achillea fragrantissima* produced a significantly reduction in the severity and frequency of diarrhea produced by castor oil. Additionally, these extracts significantly reduced the castor oil induced intestinal transit. An increase in intestinal transit time by atropine sulfate could also result from reduced in gastric emptying (Galvez *et al.,* 1996). Castor oil is also reported to induce diarrhea by increasing the volume of intestinal content by preventing the re--absorption of water. Thereby, preventing the reabsorption of NaCl and water . Probably, extracts increasing re-absorption of NaCl and water by decreasing the intestinal motility as observed by decreasing the intestinal transit by charcoal meal. The antidiarrheal activity of the extracts may be also due to the presence of high concentrations of tannins and flavonoids reported at this study. The antidiarrheal activities of medicinal plants were found to be due to tannins, alkaloids, saponins and flavonoids (Kubacey *et al.,* 2012). These ingrediants are known for inhibiting autacoids and prostaglandin, thereby inhibiting the motility and secretion. Flavonoids are natural products, which exhibit various pharmacological effects (Mata *et al.,* 1997). Quercetin, one of the flavonoids isolated from aerial parts of *Conzya flaginoides*, caused a concentration dependent inhibition of spontaneous contractions of rat ileum (Gilani *et al.,* 1994). and showed anti diarrheal activity against castor oil-induced diarrhea in mice. It also exerted inhibitory effects on guinea pig ileum contractile response (Serrano *et al.,* 2009). Rutin, another flavonoid in *Artemisia scoparia*, was found to cause a concentration dependent inhibition of spontaneous movements of rabbit jejunum (Chung et al., 1998). Besides, tannins extracted from many plants also have exhibited an anti diarrheal activity (Table 1). Tannins, a unique group of phenolic metabolites with the property of precipitating proteins, are commonly found in plants such as apple fruit, pine bark, grape seed, tea, oak, and medicinal plants and possess a variety of biological effects, including anticarcinogenic, antimutagenic, antimicrobial, and antioxidative activities (Sun *et al.,* 2009). The mechanisms of their antidiarrhoeal activity are those by inhibiting cystic fibrosis transmembrane conductance regulator protein chloride channels and by generating protein-precipitating reaction to the gastrointestinal mucosa due to the protein precipitating action (Aziz *et al.,* 2012). For evaluation of relationship between antidiarrheal activity and the total content of tannins and flavonoids in the studied extracts, the results showed a clear positive relationship between the highest total content of tannins and flavonoids and the strongest antidiarrheal only for *Achillea fragrantissima* and *Artemisia herba.* The opposite correlation (small total content of tannins and flavonoids and weak antidiarrheal activity was observed for *Rhanterium epapposum. Echinops spinosus* and *Echinops hussoni.*

Studies have been done previously on some of these plants and related plant species are agree with our results. The essential oil extracted from *Artemisia herba-alba* aerial parts reversibly relaxed the spontaneous tonus of the rabbit jejunum in a reversible concentration dependent manner with an IC_{50} value of 97.33 ± 2.59 ng/ml and reversed the tonic contraction of rat jejunum induced by KCl and carbachol (Abdalla *et al.,* 1994). Flavone cirsimartin, which is isolated from *Artemisia judaica*, *Artemisia capillaris*, *Artemisia xerophytica* and *Artemisia scoparia* is responsible for the spasmolytic activity of isolated guinea pig ileum (Harborne and Williams, 2000). Four flavones with spasmolytic activity were isolated from the aerial parts of *Artemisia abrotanum*, which are the active principles for smooth muscle relaxing activity of the plant (de la Puerta and Herrerra, 1995). The essential oil of *Achillea ageratum* L. was found to be an effective spasmolytic agent capable of inhibiting ACh and $BaCl_2$ induced contraction of isolated rat duodenum and the leaves extract has also showed anti diarrheal effect against castor oil induced diarrhea in mice (Karamenderes and Apaydin, 2003). The essential oils of *Artemisia thuscula Cav.* flowers and *Artemisia alba* were investigated and shown to have dose-dependent and essentially non-competitive spasmolytic effects in guinea pig ileum (Perfumi *et al.,* 1995). It could be concluded that, the remarkable anti diarrheal effects of *Achillea fragrantissima* and *Artemisia herba* attest to their utility in a wide range of stats of diarrhea and a further studies are required to isolate a more active constituents in these plants.

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Conflict of interest

The authors have declared that there is no conflict of interest related to this paper.

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