ANTIDIARRHEAL AND ANTIOXIDANT POTENTIAL OF FOUR BANGLADESHI MEDICINAL PLANTS

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Summary

M. indicia bark extract significantly ($p<0.01$, $p<0.001$) inhibited the mean number of defecation which were 46.3% and 49.5% at the doses of 200mg/kg and 400mg/kg respectively. Protection of the severity of diarrhea induced by castor oil was also observed. The latent period was increased and number of stools for the extract treated group was significantly ($p<0.001$) decreased as compared to control group. The result was found comparable to the effect of standard anti-diarrheal drug loperamide (55.8% at the dose of 1mg/kg). Similarly with *T. divaricata* extract percent inhibition was found to be 52.6% at the dose of 200mg/kg and 54.7% mg/kg body weight. The ethanolic extract of *K. pinnata* at the dose of 200mg/kg body weight produced 40.0% inhibition of defecation and that of at the dose of 400mg/kg body weight was 51.6% and the results with *A. calamus* extract were 24.2% and 37.9 % respectively. From the data it was also revealed that the latent periods were increased and number of stools in 4hrs for all extracts were significantly ($p<0.001$) decreased as compared to control group. The ethanolic extract of *K. pinnata* showed the highest radical scavenging activity having IC_{50} value of 67.5µg/ml followed by *T. divaricata*, *M. indica* and *A. calamus* extract having IC_{50} value of 74.5, 75.5 and 82.0µg/ml respectively. On the other hand the antioxidant activity of ascorbic acid was with IC_{50} value of 41.2µg/ml.

Key words: *Mangifera indica* L., *Tabernaemontana divaricata* L. , *Kigelia pinnata* L., *Acorus calamus* L. Antidiarrheal, Antioxidant.

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Introduction

According to World Health Organization more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance[1]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds[2].

Diarrheal diseases are one of the leading causes of morbidity and mortality in developing countries and are responsible for the death of millions of people each year[3]. Despite immense technological advancement in modern medicine, many people in the developing countries still rely on the healing practices and medicinal plants for their daily health care needs[4]. Therefore, the World Health Organization encouraged studies for the treatment and prevention of diarrheal diseases depending on traditional medical practices[5].

The people of developing countries depend on plant resources mainly for herbal medicines, food, forage, construction of dwellings, making household implements, sleeping mats, and for fire and shade. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries[6,7]. Many enzymes and secondary compounds of higher plants have been demonstrated in *in vitro* experiments to protect against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species[8]. Phenolic compounds, ubiquitous in plants are an essential part of the human diet, and are of considerable interest due to their antioxidant properties. Fruits, vegetables and beverages are the major sources of phenolic compounds in the human diet[9].

Mangifera indica L. (Family: Anacardiaceae), *Tabernaemontana divaricata* L. (Family-Apocynaceae), *Kigelia pinnata* L. (Family: Bignoniaceae) and *Acorus calamus* L. (Family: Araceae) were selected for this project. The plants possess a wide range of medicinal properties. *M. indica* has anthelmintic, antipyretic, antidiarrheal properties and used as astringent, antiscorbutic, stimulant, tonic and in toothache and debility of the stomach, ophthalmia, eruptions, diphtheria, rheumatism and catarrh of the bladder. The Bark is astringent and used in diphtheria, rheumatism, diarrhea and abdominal pain in some areas of Bangladesh. Root is used as emmenagogue, aphrodisiac, tonic to brain, liver and spleen. The juice of the leaves of *T. divaricata* is given in paralysis, urinary disorders, diarrhea, strangury and toothache[10]. The aqueous extract of stem bark of *K. pinnata* is traditionally used to heal wounds and burns, as cough suppressant and in diarrhea and dysentery by the people of Bangladesh. An infusion of the root of *A. calamus* can bring about an abortion whilst chewing the root alleviates toothache. It is a folk remedy for arthritis, cancer, convulsions, diarrhea, dyspepsia, epilepsy etc[11].

Materials and methods

Plant materials: The plants and their parts to be used for this study were selected based on their traditional uses. The fresh plant parts were collected from different areas of Bangladesh. Then the plant parts were kept under a shed till dried. The plant was identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh.

Table 1: The parts of the plants used for this study

Plant species	Part collected
Mangifera indica L.	bark
Tabernaemontana divaricata L.	Leaves
Kigelia pinnata L.	Bark
Acorus calamus L.	Roots

Preparation of extract: Extraction was carried out by simple maceration process. The dried plant parts were ground and merged in ethanol. Homogenate was kept for 4 weeks at room temperature (25 ± 2°C) in extraction bottle. After 4 weeks, the mixture was filtered twice, first using sterile cotton and then Whatman-41 filter paper. Ethanol was completely evaporated at room temperature.

Screening of antidiarrheal activity: Antidiarrheal activity was tested by using Castor oil induced method in mice[12]. Fifteen Swiss albino mice were randomly divided in to four groups (n=5). Control group received only distilled water 2ml/mice, positive control group received loperamide 1mg/kg body weight as standard and test groups received the extracts at the doses of 200mg and 400mg/kg body weight. Mice were housed in separate cages having paper placed below for collection of fecal matters. Diarrhea was induced in the mice by oral administration of castor oil (1.0ml/mice). Extract and drugs were given orally 1hr before the administration of castor oil. The time for first excretion of feces and the total number of fecal output by the animals were recorded. Normal stool was considered as numerical value 1 and watery stool as numerical value 2. Percent inhibition of defecation in mice was calculated by using the following equation:
% inhibition = $\{(M_o–M)/M_o\}x100$; where, M_o = Mean defecation of control and M = Mean defecation of test sample

Screening of antioxidant activity: Antioxidant activities of the samples were determined on the basis of their scavenging potential of the stable DPPH free radical in both qualitative and quantitative assay.

Qualitative assay: A suitably diluted stock solutions were spotted on pre-coated silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and nonpolar) to resolve polar and non-polar components of the extracts.

The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved band was observed for 10min and the color changes (yellow on purple background) were noted[13].

Quantitative assay: The antioxidant activity of the extracts was determined using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay[14]. DPPH solution (0.004% w/v) was prepared in 95% methanol. The crude extracts of different plants were mixed with 95% methanol to prepare the stock solution (10mg/100ml). The concentration of the extract solutions was 10mg /100ml or 100µg/ml. From stock solution 2ml, 4ml, 6ml, 8ml & 10ml of this solution were taken in five test tubes & by serial dilution with methanol and was made the final volume of each test tube up to 10ml whose concentration was then 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml & 100µg/ml respectively.
Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing different plant extracts (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml) and after 10min, the absorbance was taken at 517nm using a spectrophotometer.
Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (10mg/100ml or 100µg/ml) of extracts. Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95% methanol was used as blank. Percent scavenging of the DPPH free radical was measured using the following equation**:**
% scavenging = $\{(A_o–A)/A_o\}x100$; where, A_o = Absorbance of Control and A = Absorbance of test Sample.

Results

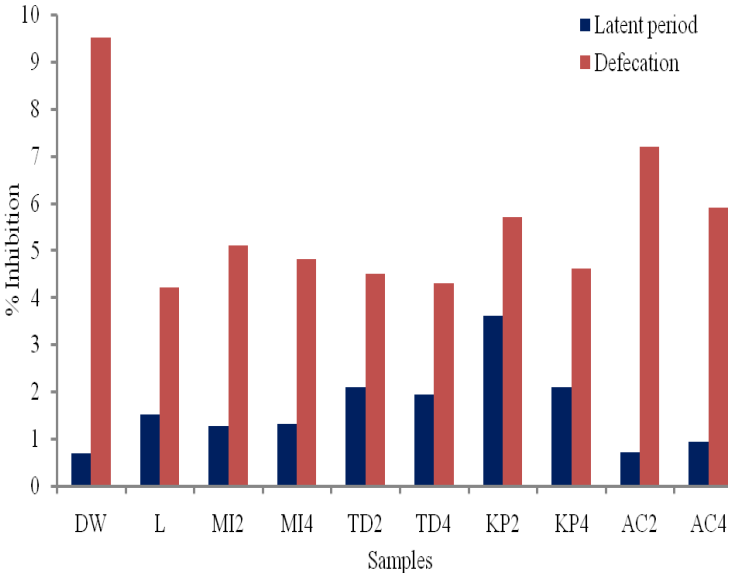
Screening of antidiarrheal activity: *M. indicia* bark extract significantly ($p<0.01$, $p<0.001$) inhibited the mean number of defecation which were 46.3% and 49.5% at the doses of 200mg/kg and 400mg/kg respectively. Protection of the severity of diarrhea induced by castor oil was also observed. The latent period was increased and number of stools at 1st, 2nd, 3rd, 4th, 5th and 6th hours for the extract treated group was significantly ($p<0.001$) decreased as compared to control group. The result was found comparable to the effect of standard anti diarrheal drug loperamide (55.8% at the dose of 1mg/kg) (Table 2).
Similarly *T. divaricata* extract showed significant ($p<0.01$) inhibition of diarrheal episodes. Percent inhibition by the extract was found to be 52.6% at the dose of 200mg/kg and 54.7% at the doses of 400mg/kg body weight. The ethanolic extract of *K. pinnata* at the dose of 200mg/kg body weight produced 40.0% inhibition of defecation and that of at the dose of 400mg/kg body weight was 51.6%. Again *A. calamus* extract produced significant ($p<0.001$) inhibition of defecation at the both doses and the results were 24.2% and 37.9 % at the doses of 200mg/kg and 400mg/kg body weight of mice respectively.
From the table 2 it was also revealed that the latent periods were increased and number of stools in 4hr for all the plants' extract were significantly ($p<0.01$, $p<0.001$) decreased as compared to control group. The result was also found to be comparable with the effect of standard antidiarrheal drug loperamide.

Table 2: Antidiarrheal activity of the samples in castor oil induced diarrheal test method on mice.

Sample	Dose	Mean ± SEM		% Inhibition
		Latent period	Defecation	
Distilled water	2 ml/mice	0.69 ± 0.08	9.5 ± 0.28	-
Loperamide	1 mg/kg	1.52 ± 0.25**	4.2 ± 0.16**	55.8%
M. indica extract	200 mg/kg	1.26 ± 0.09**	5.1 ± 0.13**	46.3%
	400 mg/kg	1.31 ± 0.61**	4.8 ± 0.21**	49.5%
T. divaricata extract	200 mg/kg	2.10 ± 0.35*	4.5± 0.32*	52.6%
	400 mg/kg	1.93 ± 0.24*	4.3 ± 0.29*	54.7%
K. pinnata extract	200 mg/kg	3.60 ± 0.35*	5.7± 0.32*	40.0%
	400 mg/kg	2.10 ± 0.24*	4.6 ± 0.29*	51.6%
A. calamus extract	200 mg/kg	0.72 ± 0.35**	7.2± 0.32**	24.2%
	400 mg/kg	0.94 ± 0.24**	5.9 ± 0.29**	37.9%

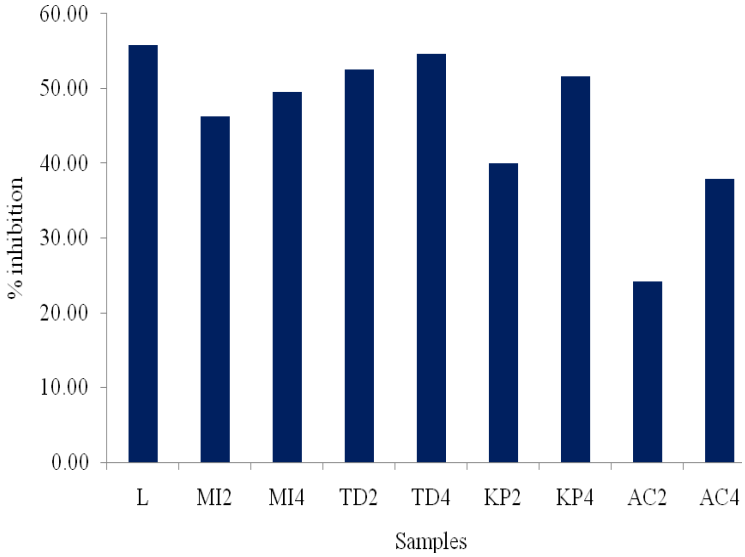
* p<0.01; ** p<0.001

Figure 1: Antidiarrheal activity of the samples in castor oil induced diarrheal test method on mice.



DW = Distilled water, L = Loperamide, MI2 = *M. indica* 200mg/kg, MI4 = *M. indica* 400mg/kg, TD2 = *T. divaricata* 200mg/kg, TD4 = *T. divaricata* 400mg/kg, KP2 =*K. pinnata* 200mg/kg, KP4 = *K. pinnata* 400mg/kg, AC2 = *A. calamus* 200mg/kg and AC4 = *A. calamus* 400mg/kg body weight

Figure 2: Percent protection of the samples in antidiarrheal test.



L = Loperamide, MI2 = *M. indica* 200mg/kg, MI4 = *M. indica* 400mg/kg, TD2 = *T. divaricata* 200mg/kg, TD4 = *T. divaricata* 400mg/kg, KP2 =*K. pinnata* 200mg/kg, KP4 = *K. pinnata* 400mg/kg, AC2 = *A. calamus* 200mg/kg and AC4 = *A. calamus* 400mg/kg body weight

Screening of antioxidant activity: The ethanolic extract of *K. pinnata* showed the highest radical scavenging activity having IC_{50} value of 67.5µg/ml followed by *T. divaricata*, *M. indica* and *A. calamus* extract having IC_{50} value of 74.5, 75.5 and 82.0µg/ml respectively. On the other hand the antioxidant activity of standard (Ascorbic Acid) was with IC_{50} value of 41.2µg/ml.

Table 3: Antioxidant activity of the samples by DPPH method

Sample	**% Inhibition**					**IC_{50} value (µg/ml)**
	20µg/ml	**40µg/ml**	**60µg/ml**	**80µg/ml**	**100µg/ml**	
Ascorbic acid	27.65%	48.54%	69.62%	73.52%	93.47%	41.2 ± 0.4
M. indica extract	13.64%	28.53%	43.26%	52.36%	67.52%	75.5 ± 0.3
T. divaricata extract	7.69%	15.86%	34.36%	56.47%	73.25%	74.5 ± 0.5
K. pinnata extract	19.56%	27.23%	42.13%	64.52%	81.25%	67.5 ± 0.4
A. calamus extract	4.32%	14.53%	21.24%	39.48%	58.37%	82.0 ± 0.4

Each value was obtained by calculating the mean of three experiments ± standard deviation.

Figure 3: Percent inhibition of the samples in DPPH scavenging assay

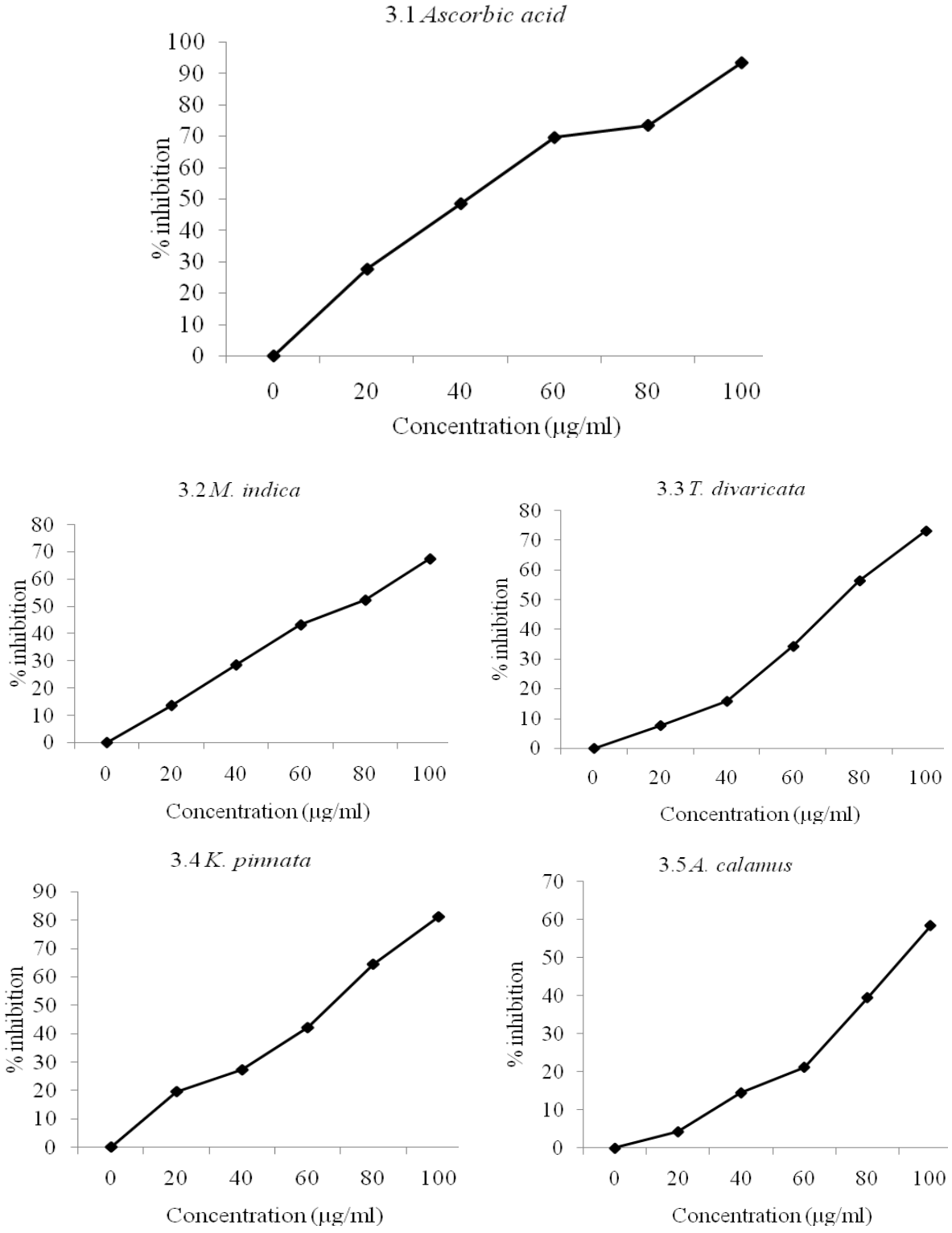
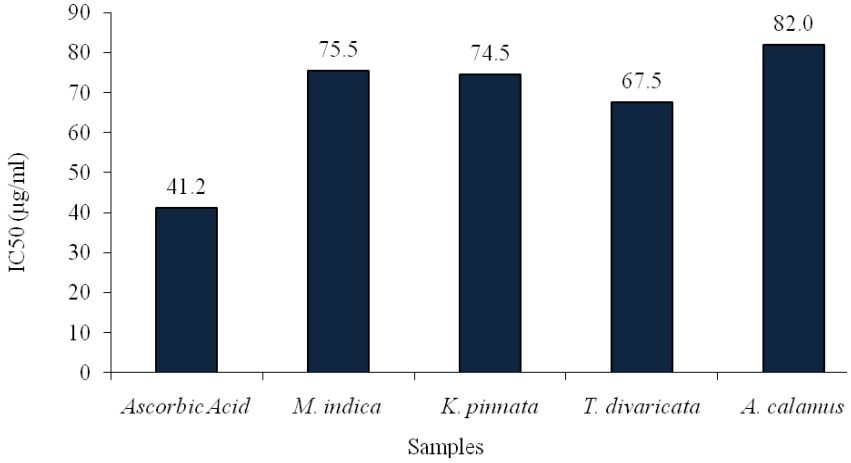


Figure 4: IC_{50} values of the plants' extracts in DPPH scavenging assay.



Discussion

Screening of antidiarrheal activity: Castor oil is made up of 90% ricinoleate[15] which is metabolized to ricinoleic acid. Ricinoleic acid causes the irritation and inflammation in the intestinal mucosa, leading to release of prostaglandins, which stimulate the net secretion of water and electrolytes into the small intestine[16,17]. We speculate that the antidiarrheal effects of the extracts may be due to the inhibition of prostaglandin biosynthesis.

Screening of antioxidant activity: The DPPH radical-scavenging capacity of the extracts could be explained by the presence of phenolic components[18]. The effect on DPPH radical scavenging was thought due to their hydrogen donating ability and radical scavenging activity[19]. As the concentration of phenolic compounds or the degree of hydroxylation of the phenolic compounds increases, so too will DPPH radical-scavenging activity[20].The polyphenol compounds had inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0g was ingested daily from a diet rich in fruits and vegetables[21]. These activities are believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[22]. In fact, many medicinal plants contain large amounts of antioxidants such as polyphenols. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases[23].

The result of this study reveals that the extracts of *M. indicia*, *T. divaricata*, *K. pinnata* and *A. calamus* contain pharmacologically active substances with antidiarrheal and antioxidant properties. The antidiarrheal effects of the extracts may be due to the inhibition of prostaglandin biosynthesis and DPPH scavenging assay may be due the presence of phenolic compounds. These plants could be a potential source of modern pharmaceutical products. Further investigation is necessary for isolation, identification and

characterization of different active compounds from the extracts and for elucidating their mode of action, responsible for these properties on different biological systems.

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References:

1. Diallo D, Hveem B, Mahmoud MA, et al. An ethnobotanical survey of herbal drugs of Gourma district, Mali. Pharmaceutical Biology 1999; 37:80-91.
2. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 2005; 4:685-688.
3. Carlos CC, Saniel MC. Etiology and epidemiology of diarrhea. Philippine Journal of Microbiology and Infectious Diseases 1990; 19: 51-53.
4. Ojewole JAO. Evaluation of antidiarrheal, antiinflammatory and antidiabetic properties of *Sclerocarya birrea* (A. Rich.) Hochst. stem bark aqueous extract in mice and rats. Phytotherapy Research 2004; 18:601-08.
5. Atta AH, Mouneir SM. Antidiarrheal activity of some Egyptian medicinal plant extracts. Journal of Ethnopharmacology 2004; 92:303-09.
6. Sandhu DS, Heinrich M. The use of health foods, spices and other botanicals in the Sikh community in London. Phytotherapy Research 2005; 19:633-42.
7. Gupta MP, Solis PN, Calderon AI, et al. Medical ethnobotany of the Teribes of Bocas del Toro, Panama. Journal of Ethnopharmacology 2005; 96:389-401.
8. Richard AL. The antioxidants of higher plants. Phytochemistry 1988; 27(4):969-978.
9. Nagendran B, Kalyana S, Samir S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chemistry 2006; 99(1):191-203.
10. Ghani A. Medicinal Plants of Bangladesh: Chemical constituents & uses, 2nd ed. Asiatic Society of Bangladesh, 2003:1-16, 381.
11. http://en.wikipedia.org/wiki/Acorus_calamus#cite_note-23.
12. Shoba FG, Thomas M. Study of antidiarrheal activity of four medicinal plants in castor oil-induced diarrhea. Journal of Ethnopharmacology 2001; 76:73-76.
13. Sadhu SK, Okuyama E, Fujimoto H, Ishibashi M. "Separation of *Leucas aspera*, a medicinal plant of Bangladesh, guided by prostaglandin inhibitory and antioxidant activities." Chemical & Pharmaceutical Bulletin 2003; 51:595-598.
14. Hasan MS, Ahmed MI, Mondal S, et al. Antioxidant, antinociceptive activity and general toxicity study of *Dendrophthoe falcata* and isolation of quercitrin as the major component. Oriental Pharmacy and Experimental Medicine 2006; 6:355-60.
15. McKeon TA, Lin JT, Stafford AE. Biosynthesis of ricinoleate in castor oil. Advances in Experimental Medicine and Biology 1999; 464:37-47.

16. Gaginella TS, Phillips SF. Ricinoleic acid: Current view of ancient oil. Digestive Diseases Science 1975; 23:1171-77.
17. Luderer JR, Dermers IM, Hayes AT. Advances in prostaglandin and thromboxane research. New York, Raven Press, 1980:1633-38.
18. Siddhuraju P, Mohan PS, Becker K. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): A preliminary assessment of crude extracts from stem, bark, leaves, flowers and fruit pulp. Food Chemistry 2002; 79:61-67.
19. Oke F, Aslim B, Ozturk S, Altundag S. Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. Food Chemistry 2009; 112:874-879.
20. Sanchez-Moreno C, Larrauri JA, Saura-Calixto F. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. Food Research International 1999; 32:407-412.
21. Tanaka M, Kuie CW, Nagashima Y, Taguchi T. Application of antioxidative Maillard reaction products from histidine and glucose to sardine products. Bulletin of the Japanese Society of Scientific Fisheries 1988; 54:1409-1414.
22. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. Journal of Agricultural and Food Chemistry 2001; 49:5165-5170.
23. Djeridane A, Yousfi M, Nadjemi B, et al. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chemistry 2006; 97: 654-660.