



Antibacterial and antidiarrheal activity of *Simarouba amara* (Aubl.) bark

Hegde Veena¹, Nagaraj Navya¹, Gowda K Sandesh², Nayaka Boramuthi Thippeswamy^{1*}

¹Department of P.G Studies and Research in Microbiology, Kuvempu University, Karnataka, India.

²Niranthara Scientific Solutions Pvt. Ltd., Bengaluru, India.

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ABSTRACT

The present study was conducted to determine the nutritional elements in *Simarouba amara* (Aubl.) bark aqueous extract (SAAE) by inductively coupled plasma optical emission spectrometry (ICP-OES) and the *in vitro* antibacterial activity against pathogens enterotoxigenic *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* by agar well diffusion, minimum inhibitory, and bactericidal concentration. Then, antidiarrheal effect was studied on castor oil-induced diarrhea in mice model. Recorded Mg > Fe > Cu > Zn elements in SAAE invariably found to be effective against Gram-positive and Gram-negative pathogens. Effective concentration of bark showed the zone of inhibition against enterotoxigenic *E. coli* (200 mg/ml), *S. typhi* and *S. aureus* (300 mg/ml), and *P. aeruginosa* and *K. pneumoniae* (100 mg/ml). The standard ratio between minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was meticulously recorded "one" against all pathogens, which confirms the bactericidal property. Results in mice model prominently showed that SAAE significantly ($p < 0.05$) reduced the frequency and number of diarrheal episodes, intestinal fluid accumulation, and intestinal transit time in dose-dependent manner. Inordinate delay in charcoal movement in the intestine positively confirmed the antispasmodic effect by reducing propulsive movement. Confirmed findings in this study naturally suggested that SAAE could be an effective antibacterial and antidiarrheal formulation.

INTRODUCTION

The normal flora of a human is limited to certain area of the organized body, including the skin, mouth, large intestine, and the vagina (Arunlakshana and Schild, 1959) may act as opportunistic pathogens in injured or immunocompromised host. According to World Health Organization (WHO), 12 families of bacteria pose the broadest threat to human health. Furthermore, these infectious organisms were divided into three ideologic categories based on the urgency of need for new antibiotics: critical, high, and medium priority. The most critical group appropriately includes multi-drug resistant bacteria such as *Acinetobacter*, *Pseudomonas*, and various Enterobacteriaceae, including *Klebsiella*, *Escherichia coli*,

Serratia, and *Proteus* sp., creates a considerable threat in hospitals, nursing homes, and among patients, whose care depends upon specialized devices like ventilators and blood catheters. They can cause severe and often deadly infections, such as bloodstream infections, diarrhea, and pneumonia. These become resistant to a massive number of preventive antibiotics, including carbapenems and third generation cephalosporins, which are available for treating multi-drug resistant bacteria. Among bacterial diseases, diarrhea remains a second leading cause of death among children under the age of five globally. Nearly, one in five children death about 1.5 million each year has been recorded. Oral rehydration solution and rotavirus vaccination recommendations by WHO in the developing countries have undoubtedly resulted decreased number of deaths by two-third, but in reality, figures are still high. As such, the remarkable progress is being gently made and much more remains yet to be done economically. In major, diarrheal disease is due to enteropathogens, diarrheagenic *E. coli* remain a most common causative agent in developing countries; Enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli*, encroaching pathogens like

*Corresponding Author

Nayaka Boramuthi Thippeswamy, Department of P.G Studies Research in Microbiology, Kuvempu University, Shivamogga, India.
Email: nbtmicro@gmail.com

Shigella spp., *Campylobacter* and *Salmonella* spp. Antimotility agents (loperamide), antisecretory agents (racecadotril), and adsorbents (diosmectite) possess dreadful side effects in children. In consequence, traditional medicines become a deliberate choice of equitable treatment. Centralized governments in India, China, and South Africa are seamlessly incorporating traditional, a less side effective health care practices into their national scheme to overcome the problems. Accomplished work on plant products in the recent years reliably provided the source to develop naturally a potent formulation to encounter diarrhea and other illness with positively enhancing the immune system (Ismail and Asad, 2009). Similarly, *Simarouba amara* (Aubl.), which is a native plant species of *Simaroubaceae* family, has been used as a natural medicine in tropics. The family includes 32 genera and more than 170 species of trees. *Simarouba amara* (Aubl.) is a medium-sized tree, indigenous to the Amazon Rainforest and other tropical areas in Mexico, Cuba, Haiti, Jamaica, and Central America. French explorers fortunately discovered and found that the ancient Indian tribes in the Guyana rainforest used *S. amara* (Aubl.) bark as an effective treatment for dysentery and malaria. In Brazil, the majestic tree is traditionally called negrito or “dysentery bark.” The excellent tonic of bark is conventionally used to wash skin sores and to treat dysentery, diarrhea, stomach and bowel disorders, hemorrhages, and internal bleeding. The active constituent “quassinoids,” which belongs to the triterpene chemical family, have been credibly reported for vast spectrum of biological activities, such as antitumor, antimalarial, antiviral, effective insecticide and feeding deterrent (Khan *et al.*, 1996; Polonsky *et al.*, 1978; Taylor, 2003; Wright *et al.*, 1988) antiparasitic, and herbicidal activities. Dehydroglucarubinone possesses antineoplastic activity by inhibiting DNA polymerase activity destructing cancer cell membrane system. It was found precisely that SA bark is effective in treatment for gastric ulcer and hepatic damage in mice (Maranhão *et al.*, 2014A). Quassinoid containing fraction of the stem has strikingly shown amebicidal and antimalarial activity against *Entamoeba histolytica* and *Plasmodium falciparum* (Maranhão *et al.*, 2014A). In the present study, the nutritional elements in *S. amara* (Aubl.) bark aqueous extract (SAAE), antibacterial activity against human pathogens, and antidiarrheal activity of SAAE was scientifically investigated in mice via an oral route.

MATERIAL AND METHODS

Chemicals

Simarouba amara (Aubl.) bark was kindly provided by Niranthara Scientific Solutions Pvt Ltd. Bengaluru, Karnataka, India, Castor oil (Cremophor®; Himedia), Loperamide Hydrochloride Capsules IP (Eldoper®; Microlabs Ltd.), Atropine sulfate monohydrate (Himedia), Charcoal-activated powder, L.R, (Himedia), and Gum Acacia powder (Himedia). Eterotoxigenic test organisms used: *E. coli* (ETEC H10407 LT⁺, ST⁺) (MTCC 723), *Salmonella enteric serovar Typhi* (MTCC 733), *Staphylococcus aureus* (MTCC 96), *Klebsiella pneumoniae* (MTCC109), and *Pseudomonas aeruginosa* (MTCC741). Stock cultures were maintained on nutrient agar slants at 4°C and sub cultured in nutrient broth at 37°C prior to each antimicrobial test.

Preparation of bark extract

Simarouba amara (Aubl.) bark aqueous extract was prepared as previously described method (Maranhão *et al.*, 2014 B). Briefly, 10-g bark powder was extracted with 100-ml distilled water (10:100 w/v) as solvent by boiling for 15 minutes. After cooling, the extract was filtered through no.1 Whattman filter paper and obtained aqueous extract was concentrated in lyophilizer and stored in air tight vials at 4°C. After drying, percentage of yield was determined.

Animals

Healthy Swiss albino female mice (20–25g b. w) were used for the experiment. Mice were acclimatized for 7 days to laboratory conditions for minimizing any nonspecific stress. Animals were fed with a standard commercial pellet feed and received clean drinking water *ad libitum*. The temperature varied between 28°C and 30°C and relative humidity of about 56%–60% with 12-hour light-dark cycle. The animals were handled according to Institutional Animal Ethics Committee (IAEC) standard guidelines in experiments (NGSMIPS/IAEC/MARCH-2018/103).

Determination of nutritional elements in plant extract by ICP-OES

Simarouba amara (Aubl.) bark aqueous extract (lyophilized) was subjected for nutritional element analysis by inductively coupled plasma optical emission spectroscopy (ICP-OES) method (Shao and Bian, 2002). Briefly, 0.25-g SAAE was digested with 5-ml concentrated nitric oxide (HNO₃) and left for 12 hours. After the pre-digestion for 12 hours, sample was digested at 180°C on a hot plate till the nitric acid about to evaporate. Furthermore, 5 ml of mixture of di-acids (nitric oxide and perchloric acid, 10:2) was added to the sample and digested until the sample become transparent. Sample was cooled at the room temperature and filtered through filter paper in volumetric flask (25 ml) and volume was made up with 2% HNO₃. Meanwhile blank without the sample was also processed in the similar manner and elements (Mg, Cu, Fe, and Zn) were analyzed through ICP-OES (Perkin Elemer-5300 V).

Determination of antimicrobial activity by well diffusion method

Antimicrobial activity of bark aqueous extract was evaluated by well diffusion method (Bennett *et al.*, 1996). Bacterial cultures were adjusted to 0.5 McFarland turbidity standards. Equal volume (100 µl) of different concentration of reconstituted bark extract (100, 200, 300, 400, and 500 mg/ml), and standard antibiotics (Co-Trimaxazole, Ampicillin, and streptomycin; 1 mg/ml) were used. Furthermore, the zone of inhibition (mm) was measured against each pathogen.

Determination of MIC and MBC by broth dilution method

Two-fold serial dilutions were made with Muller Hinton broth from reconstituted bark extract (10–1.25 mg/ml) and 100 µl/well from all dilutions used. Nearly, 100-µl bacterial inoculum (10⁵ CFU/ml) was added to respective wells containing the test plant extract except negative control and mixed and incubated for 24 hours. After incubation, 50-µl MTT dye [3-(4, 5 –Dimethylthiazol-

2-yl)-2, 5-diphenyl tetrazolium bromide; yellow dye; 0.020 mg/ml] was added to each well and incubated for 30 minutes at 37°C. The viable bacterial cells in the well change the yellow MTT dye to blue color. In assay, the lowest concentration at which no visible colour change was observed and that was considered as minimum inhibitory concentration (MIC) (Elena *et al.*, 2003). Similarly, the minimum bactericidal concentration (MBC) was determined by sub culturing the test dilution on fresh solid medium and further incubated at 37°C for 18–24 hours. The lowest concentration of MIC tubes with no visible bacterial growth on solid medium was considered as MBC (Bennett *et al.*, 1996; Wright *et al.*, 1988).

Effect of SAAE on castor oil-induced diarrhea in mice

The experiment was performed according to previously described method (Degu *et al.*, 2016). Briefly, 25 mice were fasted for 18 hours and randomly divided into five groups of five mice each. Further, mice in group I (control) received sterile water 1 mL/100 g p.o., group II (positive control) received loperamide 3 mg/kg p.o., and mice in groups III, IV, and V SAAE (100, 200, and 300 mg/kg, p.o.) in single dose, respectively. After 60 minutes, 0.5-ml castor oil was given orally to animals in all groups. Each animal was placed in individual cage, whose floor was lined with blotting paper and changed every hour for observation up to 4 hours. While observing, time of onset of diarrhea, total number of stools, and number of wet feces excreted by animals were recorded, and the percentage of inhibition of defecation was calculated by using the following formula:

$$A = \text{Average number of wet feces in control group}$$

$$B = \text{Average number of wet feces in test group.}$$

Effect of SAAE on gastrointestinal transit

This was evaluated by charcoal meal marker diet test (Brijesh *et al.*, 2009; Degu *et al.*, 2016; Sharma *et al.*, 2009). Swiss Albino mice were randomly divided into five groups of five mice each, fasted for 18 hours with free access to water. The group I (control) mice were administered with sterile water 1 ml/kg p.o., group II (positive control) with atropine sulfate 2.5 mg/kg i.p., and groups III, IV and V with SAAE 100, 200, and 300 mg/kg, p.o. in single dose, respectively. Thirty minutes after drug administration, mice in all groups were fed with 1-ml charcoal meal by gastric gavage (10% activated charcoal suspension in 5% gum acacia) as a food tracer. All mice were sacrificed by cervical dislocation after 30 minutes and dissected longitudinally. Total length of small intestine and charcoal movement from pylorus to caecum was measured by calibrated ruler. Percentage of inhibition and peristalsis index was expressed by using the following formula:

$$A = \text{Distance travelled by charcoal meal in control group (cm)}$$

$$B = \text{Distance travelled by charcoal meal in treated group (cm).}$$

Effect of SAAE on castor oil-induced enteropooling

Mice were randomly divided into five groups of five mice each, fasted for 18 hours with free access to water. The group I (control) mice were treated with sterile water 1 ml/kg p.o., group II (positive control) with loperamide 3 mg/kg, p.o., and the groups III, IV, and V with SAAE 100, 200, and 300 mg/kg, p.o. in single dose, respectively. After 30 minutes, mice in groups

were challenged with 1 ml of castor oil orally. After 30 minutes, mice were sacrificed by cervical dislocation; small intestine was excised from pylorus to caecum. Immediately, small intestine was weighed. Contents of intestine were collected by milking into a graduated tube and the volume was measured. Eventually small intestine was reweighed, the difference between full and empty intestine was calculated. Percentage of inhibition of fluid accumulation and percentage inhibition of intestinal content was determined (Ngo-Teke *et al.*, 2010).

$$\text{MVICC} = \text{Mean volume of intestinal content in control group}$$

$$\text{MVICT} = \text{Mean volume of intestinal content in treated group}$$

$$A = \text{Mean weight of intestinal content in control group}$$

$$B = \text{Mean weight of intestinal content in treated group.}$$

In vivo antidiarrheal index

Antidiarrheal index was expressed after cumulating the data according to the formula:

$$\text{In vivo antidiarrheal Index (ADI in vivo)} =$$

D_{frq} (%) is the delay in defecation time (minutes), G_{MT} = Gut meal travel reduction (as % of inhibition), and P_{frq} = the reduction in the number (frequency) of stools (Degu *et al.*, 2016).

Statistical analysis

Analysis was performed using Graph Pad Prism version 5.0 (GraphPad Software, La Jolla, CA). A result in antibacterial activity was a minimum of three repetitions and analyzed using standard error mean. In antidiarrheal activity, difference between groups was analyzed with one way analysis of variance (ANOVA) and $p < 0.05$ was considered as statistically significant. Error bar represents mean with standard deviation.

RESULTS AND DISCUSSION

Present task to carefully develop a convenient antibiotic is a time-dependent process and may severely hinder the effective treatment option. Therefore, attempts must be wisely directed toward the necessary development of the effective natural, non-toxic drug for restorative treatment (Chandra, 2013; Kang *et al.*, 2011). Various published findings on secondary metabolites in distinct plants delivered the rich resource to sufficiently develop a novel compound to encounter drug-resistant pathogenic bacteria and diarrhea causing organisms (Khan *et al.*, 2012).

Nutritional elements in SAAE by ICP-OES

The medicine value of a plant depends on its macro and trace elements. Nitrogen, phosphorous, magnesium, potassium, calcium, sulphur, carbon, oxygen, and hydrogen are macro elements, whereas iron, copper, chlorine, zinc, boron, and molybdenum are considered as trace elements. In our ICP-OES element analysis experiment, SAAE showed higher amount of magnesium (3240.6 µg/g), iron (318.4 µg/g), copper (315.3 µg/g) than zinc (10.1 µg/g) (Table 1). These nutritional elements have complex interaction with in a system and important to prolong the normal metabolism (Alberts *et al.*, 1995; Raj *et al.*, 2017). Moreover, certain trace elements are low in human body and are essential for normal functions. Na⁺, K⁺, and other macro elements are playing a major role in osmotic pressure and acid base

Table 1. Available nutritional elements in SAAE by ICP-OES method.

Test sample	Cu ²⁺	Mg ²⁺	Fe ²⁺	Zn ²⁺
	(µg/g)			
SAAE	315.5	3240.6	318.4	10.1

balance. But, Mg²⁺ deficiency results in increased excitability of the nervous system and hence key element for proper functioning of central nervous system. Then, Fe²⁺ is a carrier of hemoglobin and an active site of many enzymes. In contrast, Zn²⁺ and Cu²⁺ are important for immunity and involved in acceleration of healing of damaged tissue, resistance over infections (Kiel *et al.*, 2016; Qing-hua *et al.*, 2012; Zeng *et al.*, 2001). Thus, the study of elements in our bark extracts reveals the mechanism involved in its antibacterial and antidiarrheal property.

Antibacterial activity by well diffusion method

Novel antibiotics slow down the possible infection by disrupting the complex synthesis of the peptidoglycan layer constructing the bacterial cell wall. Similarly, phenols, flavonoids, and alkaloids kill the infectious microorganisms. Notable core lipophilic active component Curcumin-I was reported in *Curcuma longa* as a polyphenol. This leads to membrane leakage in gram positive (Methicillin-resistant *S. aureus* and *Enterococcus faecalis*) and gram negative (*E. coli* and *Pseudomonas aeruginosa*) bacteria. Meanwhile, the curcumin-I showed bactericidal activity at minimum load (10⁴ CFU/ml) and exerted bacteriostatic mechanism at maximum load (10⁶ CFU/ml) of organisms (Aqil *et al.*, 2005; Tyagi *et al.*, 2015). In the present study, SAAE appreciably reduces the intended load of *P. aeruginosa* and *K. Pneumoniae* (100 mg/ml), ETEC H10407 (200 mg/ml), and *S. typhi* and *S. aureus* (300 mg/ml) (Fig. 5 A–Y). Meanwhile, standard antibiotic co-trimoxazole is mean for treating *E. coli* infections but shown moderate zone of inhibition against ETEC and shown maximum antibacterial activity *S. aureus* and minimum or nil at both *K. pneumonia* and *P. aeruginosa*. Similarly, ampicillin had shown moderate activity against *S. typhi* and maximum against *S. aureus*. But, streptomycin a broad spectrum antibiotic had shown antibacterial activity against all test pathogens. Comparative results in our study had shown that SAAE can compete with the tested synthetic antibiotics. Since as reported explained findings on active component in species of *Simaroubaceae* family suggested the inhibition of protein synthesis and aerobic respiration in microorganisms. Likely results observed in our study (agar well diffusion method) positively obey the stated mechanism (Table 2) (Valle *et al.*, 2015). Moreover, the presence of Zn²⁺, Mg²⁺, Cu²⁺, and Fe²⁺ in SAAE extract increased its antibacterial efficacy. According

to the studies, Zn binds to the membrane of organism and interferes in cell division by extending the lag phase in cell cycle (Lemire *et al.*, 2013). Similar effect was observed in Mg²⁺ and Ca²⁺ in antibiotic resistant *S. aureus* eradication. Both were involved in membrane disruption and kill the *S. aureus* at stationary phase (Arakha *et al.*, 2015; Weaver *et al.*, 2014; Xie and Yang, 2016).

MIC and MBC

SAAE typically exhibited the bactericidal nature by showing precisely MBC/MIC ratio “1.” The used concentration in MIC and MBC appeared high against selected pathogens (Banfi *et al.*, 2003; Nemeth *et al.*, 2015) (Table 2), but the beneficial activity of concentrated extracts is unrelated to their respective dry weight. Observed result in studies, for instance clove (95 mg/ml), guava (122 mg/ml), and garlic (133 mg/ml) extracts, showed alike antimicrobial activity patterns (Satyajit and Lutfun, 2007). Considered results in our present study represent an agreement with several previous findings, the gracious presence of cardiac glycosides, terpenoids, flavonoids and phenols accurately reflect the membrane leakage, chief hindrance in protein synthesis and aerobic respiration in pathogens.

Effect of SAAE on castor oil-induced diarrhea in mice

Castor oil is laxative agent in which ricinoleic acid, a hydroxylated fatty acid, released from castor oil by intestinal lipases (Tunaru *et al.*, 2012). Consequently, it activates an EP3 prostanoid receptor that mediates the elevated production of prostaglandins, nitric oxide, which produces local irritation, inflammation, net secretion of water, and electrolytes in lumen. Thoroughly documented fact on infectious diarrhea confirms the multifactorial and series of cascading reactions, fundamental imbalance in electrolyte absorption (active Na⁺ and K⁺), and smooth muscle contractility in the intestine via inhibiting Na⁺-K⁺ ATPase in the small intestine (Enzo, 2006). In the present study, result showed, the standard dose of castor oil administered was sufficient to naturally evoke the diarrhea. SAAE was assuredly found to be effective in a dose-dependent 100, 200, and 300 mg/kg b. w manner. At an active dose of 300 mg/kg, bark extract reduced the number of wet feces and progressively extended the latency phase up to 195.33 minutes and onset of diarrheal episode (Fig. 1) significantly (*p* < 0.05). Overall at 300 mg/kg, bark extract reduced (42.85%) the castor oil-induced diarrhea compared to reference drug loperamide (33.42%). The findings in this study are in line with previous likely studies and SAAE acts like non-steroidal anti-inflammatory drug. Consequently, it inhibits the prostaglandin production and slows down the diarrhea.

Table 2. Antibacterial activity of SAAE against infectious bacterial pathogens; “0”: No inhibition zone was observed, Std: Co-Tr: Co-Trimaxazole, Am: Ampicillin St: Streptomycin, ETEC: Enterotoxigenic *E. coli* H10407 (LT⁺, ST⁺), *S. typhi*, *K. pneumoniae*, *Pseudomonas aeruginosa*, and *S. aureus*

Test Organisms	Zone of Inhibition (mm)										
	Concentration (mg)										
	25	50	75	100	200	300	400	500	Co-Tr	Am	St
ETEC H10407	0	0	12.6 ± 0.33	18.3 ± 0.33	22.66 ± 0.33	21.6 ± 0.33	11.6 ± 0.33	14.6 ± 0.33	26.3 ± 0.33	21 ± 0	24 ± 0
<i>S. typhi</i>	6.6 ± 0.33	12 ± 0.33	11.6 ± 0.33	16.66 ± 0.33	18.33 ± 0.44	19.33 ± 0.33	14 ± 0	16.66 ± 0.33	39.33 ± 0.33	21.33 ± 0.33	26 ± 0
<i>S. aureus</i>	0 ± 0	0 ± 0	0 ± 0	13.3 ± 0.33	14.6 ± 0.33	15.3 ± 0.33	11.3 ± 0.33	12.6 ± 0.33	40 ± 0	31 ± 0	25.33 ± 0.33
<i>K. pneumoniae</i>	0 ± 0	0 ± 0	12.6 ± 0.33	15.3 ± 0.33	13.33 ± 0.33	14 ± 0	11.6 ± 0.33	13 ± 0	0 ± 0	18.66 ± 0.33	26.66 ± 0.33
<i>P. aeruginosa</i>	0 ± 0	0 ± 0	11.66 ± 0.33	16 ± 0	14.66 ± 0.33	15 ± 0	13 ± 0	13.3 ± 0.33	0 ± 0	23 ± 0	26 ± 0

Table 3. MIC and MBC of SAAE against infectious bacterial pathogens

Organism	MIC	MBC	MBC/MIC ratio	Activity
	(mg/ml)			
ETEC	10	10	1:1	Bactericidal
<i>S. typhi</i>	10	10	1:1	Bactericidal
<i>S. aureus</i>	5	5	1:1	Bactericidal
<i>K.pneumoniae</i>	10	10	1:1	Bactericidal
<i>P. aeruginosa</i>	5	5	1:1	Bactericidal

SAAE produced significant reduction at a lower dose (300 mg/kg) compared to previously reported antidiarrheal plant extract like methanol and chloroform leaf extract of *Croton macrostachyus* hochsht. ex Del. produced overall decline in castor oil-induced diarrhea at 300, 400, and 500 mg/kg and aqueous extract at 1,000 mg/kg (Degu *et al.*, 2016). Investigative findings on tannins, terpenoids, flavonoids, and other secondary metabolites suggested their opioid like mechanism in silencing the direct action on secreto-motor neuronal pool in the submucosal plexus. This results in the reduced fluid secretion in a small intestine and dried, harder stool in the large intestine.

Effect of SAAE on gastrointestinal motility test

Ricinoleic acid is gently released by the castor oil absorbed body via intestinal mucosa where prostaglandin EP3 receptors are present. This acts upon G-proteins and sequentially on muscle cells of the intestine, which naturally induces pain. Further, it is responsible for increased propulsive movement. Mainly muscarinic acetylcholine receptors M1 and M2 preferentially bind to Gq protein and Gs/Gi protein, which act on secondary messengers like up regulation of phospholipase, down regulation of cyclic adenosine monophosphate (cAMP),

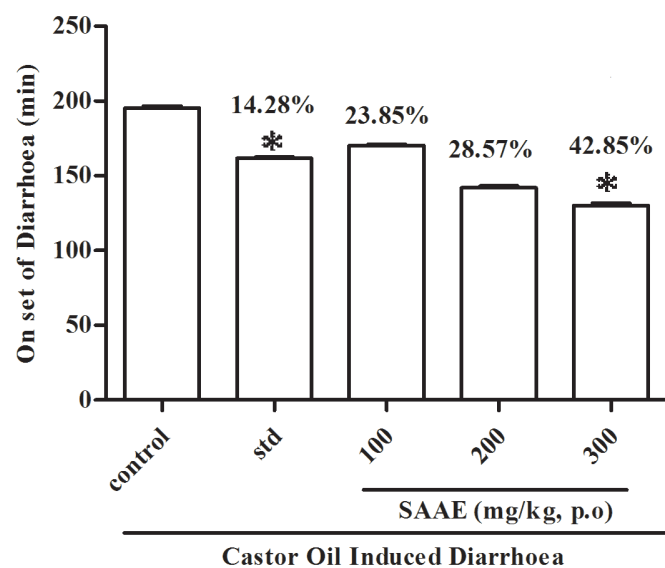


Figure 1. SAAE extract administration reduced the onset of diarrhea (min) in dose-dependent manner and loperamide (3 mg/kg, p.o) used as standard. Data were expressed as Mean \pm SD ($n = 5$). Analysis was performed with One-Way ANOVA. The asterisk* stands for $p < 0.05$ compared with model control (castor oil-induced diarrhea). Superscripts were the protection percentage of diarrhea in each dosage group.

and important physiological role in properly regulating the peristaltic movements of the gut (Brown and Taylor, 1996). In our completed study, SAAE has significantly lessened the gastrointestinal motility or charcoal meal movement accordance with dose (100, 200, and 300 mg/kg b.w) dependent manner ($p < 0.05$) (Fig. 2). At the dose 300 mg/kg, bark extract has sufficiently reduced the peristaltic movement and decreased intestinal spasms by inhibiting the parasympathetic activity. Standard drug atropine and other anticholinergics act as an “encephalin”—worthy antagonist on muscarinic receptors and decrease the intestine motility by inhibiting acetylcholine. Further helps in delaying of intestine peristaltic movement and gastric emptying (Arunlakshan and Schild, 1959; Maddison *et al.*, 2002). Peer-reviews on studies of flavonoids and polyphenols have describe the able results to inhibit the prostaglandin EP3 activated muscle contraction and inhibiting the unconditional release of prostaglandins and autocooids (Brijesh *et al.*, 2009; Dosso *et al.*, 2011). Accordingly, results in our experiment revealed the possible role in gastrointestinal motility. SAAE is enriched with flavonoids, phenols and Ca^{2+} was acts as better antagonist and reduces the intestinal movement via calcium sensing receptor (CaSR) activation (Favus *et al.*, 1981). Moreover, Ca^{2+} stimulates epithelial cell differentiation and maturation, increased gastrin-hormone and Ca^{2+} dependent CaSR activation modulates the intestine fluid secretion by inhibiting cyclic nucleotides [cAMP/cyclic guanosine monophosphate (cGMP)] and increases the nutrient contact time for absorption by intestine villi (Kirchhoff and Giebel, 2006). The fact of malabsorption and micronutrients deficiency prolongs the diarrhea and clinical damage. Hence, the Indian Academy of Pediatrics suggests the micronutrients uptake

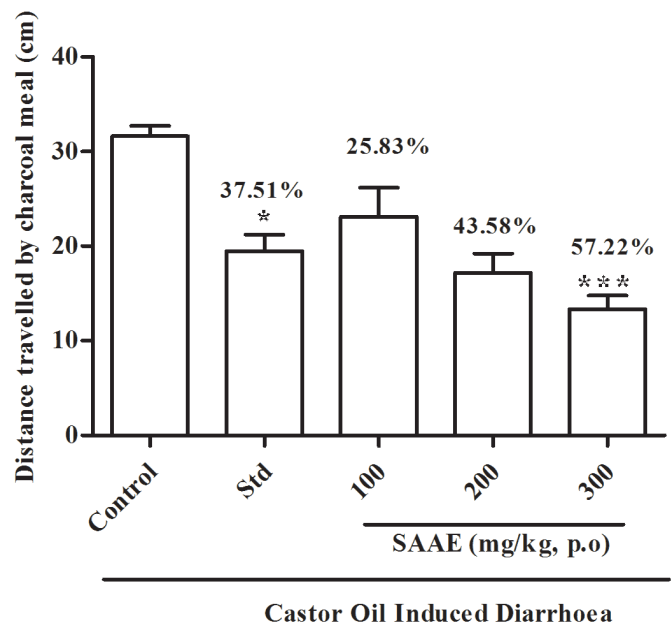


Figure 2. SAAE administration reduced the charcoal movement in small intestine in dose-dependent manner and atropine sulfate (2.5 mg/kg, i.p) used as standard. Data were expressed as Mean \pm SD ($n = 5$). Analysis was performed with One-Way ANOVA. The asterisk* stands for $p < 0.05$ compared with model control (castor oil-induced diarrhea). Superscripts were the percentage of inhibition of gastro intestinal motility in each dosage group.

for 2 weeks in treatment of diarrhea along with rehydration therapy. Major nutrients are folic acid (5 mg day 1, then 1 mg/day), zinc (2 mg/kg/day), and copper (0.3 mg/kg/day), and vitamin A, magnesium 0.2 ml/kg/dose twice, and 3 mg/kg/day of iron (Bhutta *et al.*, 2000). The folic acid, copper, selenium, vitamin A, and zinc help in restoring small intestine damage (Giannattasio *et al.*, 2016). This favorable result clearly supports the proper evidence to its local name “dysentery bark” and antidiarrheal property meaningfully compared with standard drug loperamide and atropine sulfate.

Effect of SAAE on castor oil-induced enteropooling

In our attentive study, SAAE was convincingly showed antidiarrheal property by reducing intestinal fluid accumulation at doses 100, 200, and 300 mg/kg significantly ($p < 0.05$) (Fig. 3). In established physiology, endogenous nitric oxide is proabsorptive and it influence on enteric nervous system, suppress the prostaglandin formation, and opening of basolateral K^+ channels. Moreover, nitric oxide synthase inhibitors (e.g., l-NG-nitro-arginine methyl ester, l-NAME; NG-monoethyl-arginine, NMMA; NG-nitro-arginine, NNA; 7-nitroindazole; s-methylisothiourea, SMT etc.) lead to net secretion by generating prostaglandin in animal model such as mice, rats, guinea pigs, rabbits, and dogs (Adeyemi and Akindele, 2008). Hence, in the presence of a laxative agent, nitric oxide synthase produced more elevated concentrations that evoke net secretion. SAAE reduced the fluid accumulation efficiently at 300 mg/kg by inhibiting the nitric oxide, and consequently prostaglandin E2 production. Alkaloids (Enzo, 2006), flavonoids (Hamalainen *et al.*, 2011; Medina *et al.*, 1997), and terpenoids (Maciel *et al.*, 2000) inhibit the intestinal motility and hydroelectrolytic secretions, whereas, tannins denature functional proteins in the intestinal mucosa by

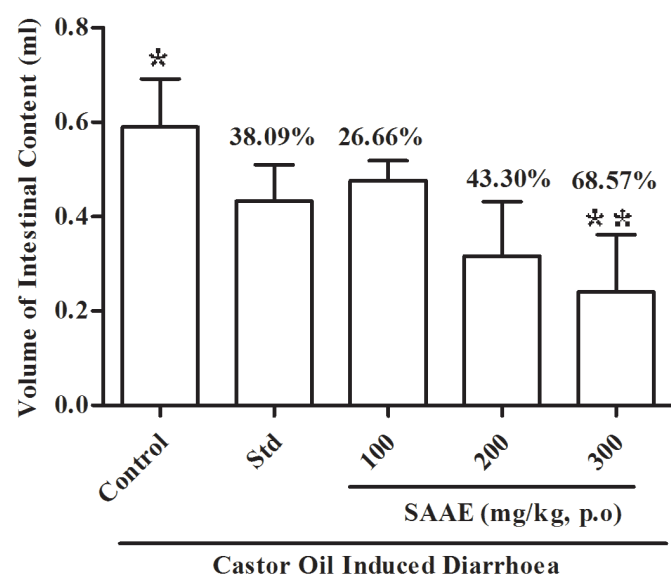


Figure 3. SAAE administration reduced the intestinal fluid accumulation in dose-dependent manner and loperamide (3mg/kg, p.o) used as standard. Data were expressed as Mean \pm SD (n = 5). Analysis was performed with One-Way ANOVA. The asterisk* stands for $p < 0.05$ compared with model control (castor oil-induced diarrhea). Superscripts were the percentage of inhibition of accumulation fluid content in small intestine in each dosage group.

forming protein tannates, which adequately reduces the fluid secretion by inhibiting nitric oxide synthase (Almieda *et al.*, 1995; Tripathi, 2008; Yadav and Tangpu, 2007). Potentially, tannins are involved in silencing the EP3 receptor on mucosal layer, which activate calcium pumping system (which induces the muscle relaxation). Most of terpenoids like abietic acid and steroids like phytosterols reported for the inhibition of prostaglandin E2 production, which reregulates the propulsive movement of gut (Awad *et al.*, 2004). Moreover, synergistic action of terpenoid and flavonoid acts against inflammation and counteracts the complex NO synthesis. At present, two classes of antidiarrheal synthetic drugs; opioids, adsorbent and bulking agent are preferentially used for treatment. Loperamide is an opioid, a mainstay in successful treatment, which binds to μ -receptor on neurons in the sub mucosal neural plexus of the small intestine. The instantaneous action on μ -receptor prolongs the transit time and anti-motility action enhances segmental contraction of the colon, thereby inhibiting small intestine contraction colonic action. SAAE remarkably exerted the therapeutic effect with 60.46% protection percentage. Taking everything into account, the recorded available secondary metabolites and nutrients (Mg, Fe, Cu, and Zn) of SAAE may increases the $Na^+ - K^+$ ATPase enzyme activity by reducing the concentration of nitric oxide in small intestine. Mg^{2+} and Cu^{2+} ions are fundamental component of the body, which help in stabilizing the enzymes and strike out the cramps of muscle during its deficiency. Cu^{2+} ions are critical for metalloenzymes such as superoxide dismutase, which neutralizes the potential damaging superoxide anions, nitric oxide radical, oxidative stress, and oxidative damage (Ighadaro and Akinloye, 2018). The first line defensive grid of antioxidant enzyme ruled by metal ions in SAAE succeeds in nitric oxide accumulation and other consequent diarrheal damages. Contrarily, Fe^{2+} is essential for enzymes, involved in energy production, transport of oxygen in blood, and boosting of immune function. Besides that, Zn^{2+} is critical for 300 enzyme activities and essential for carbohydrate metabolism. Its antioxidant property helps in healing and regeneration of damaged tissues during diarrhea.

In vivo antidiarrheal index

In vivo antidiarrheal index of SAAE was observed in dose-dependent way (Fig. 4). The highest antidiarrheal index was keenly observed at the effective dose 300 mg/kg. The

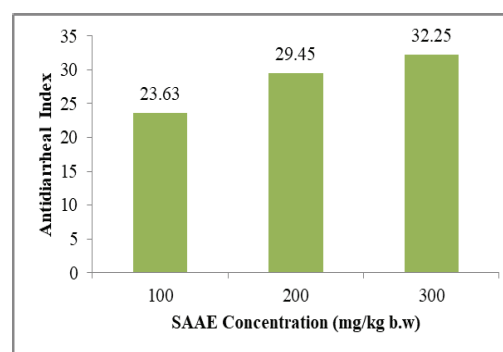


Figure 4. *In vivo* antidiarrheal index (ADI) of SAAE. Bark was reduced the overall symptoms of castor oil-induced diarrhea in dose-dependent manner. nem.

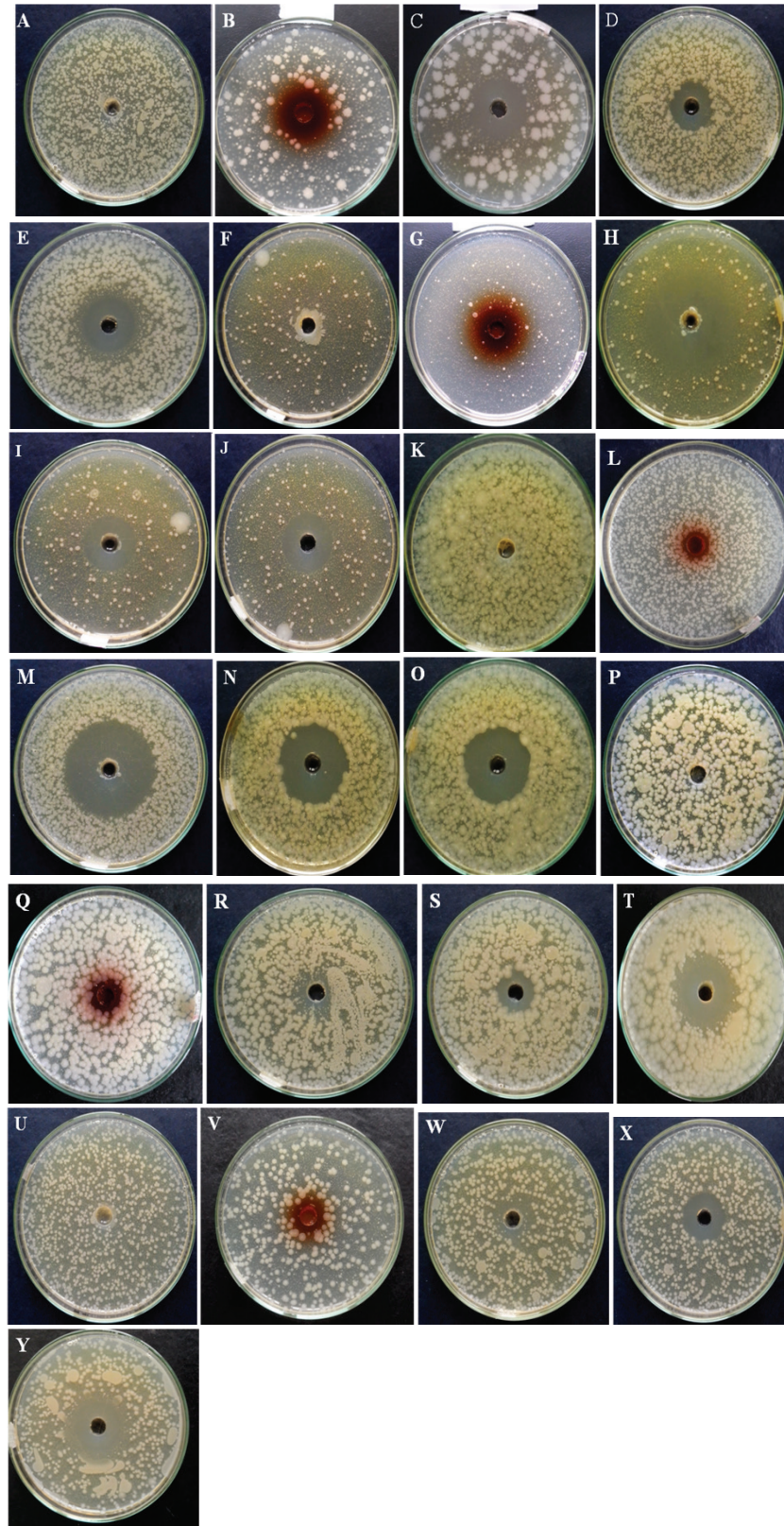


Figure 5. Antibacterial activity of SAAE against pathogen: Enterotoxigenic *E. coli* ETEC H10407 (A-E); A—control; B—SAAE (200 mg/ml); C—Co-Trimaxazole; D—Amphicillin; E—Streptomycin; *Salmonella typhi* (F-J) F—Control; G—SAAE (300 mg/ml); H—Co-Trimaxazole; I—Amphicillin; J—Streptomycin; *Staphylococcus aureus* (K-O) K—Control; L—SAAE (300 mg/ml); M—Co-Trimaxazole; N—Amphicillin; O—Streptomycin; *Klebsiella pneumoniae* (P-T) P—Control; Q—SAAE (100 mg/ml); R—Co-Trimaxazole; S—Amphicillin; T—Streptomycin; *Pseudomonas aeruginosa* (U-Y) U—Control; V—SAAE (100 mg/ml); W—Co-Trimaxazole; X—Amphicillin; Y—Streptomycin. *Co-Trimaxazole, Amphicillin and Streptomycin (1 mg/ml).

antidiarrheal index (ADI) is a measure of a combined effect of the independent parameters of diarrhea such as purging frequency, onset of diarrheal stools, and intestinal motility. Accurate representation of ADI is useful specification in ranking an antidiarrheal agent (Adeyemi and Akindele, 2008; Brijesh *et al.*, 2009; Mbagwu *et al.*, 2008; Umer *et al.*, 2013). The higher the ADI value, the more the effective against diarrhea.

CONCLUSION

Based on the above findings, the present study provides convincing evidence that SAAE possesses remarkable antibacterial and antidiarrheal activity. A noticeable presence of flavonoids, phenols, terpenoids, and micronutrients (ICP-OES sensitive method) in extract showed the protective effect and is rapid, long lasting, and statistically significant. It might be rightfully approachable formulations to provide better treatment against infectious diarrhea along with electrolyte therapy. However, further chemical and pharmacological studies are reasonably required to elucidate the precise mechanism.

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CONFLICT OF INTEREST

Authors declare that there are no conflicts of interest.

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