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ANTIDIARRHEAL AND ANTIBACTERIAL ACTIVITIES OF HYDROALCOHOLIC EXTRACTS OF *SALVIA SCHIMPERI* BENTH FROM ETHIOPIA

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ABSTRACT: In Ethiopian folk medicine, aqueous decoction of the leaves of *Salvia schimperi* is used for treatments of various ailments including diarrhea, however, to date, there appear to have been no reports on the phytochemistry, the antidiarrhoeal and the antimicrobial activity of the plant. In this study, the hydroalcoholic extract of the leaves of *S. schimperi* was evaluated for its antidiarrheal and antibacterial activities against castor oil induced diarrhea in mice and six clinically isolated enteric bacterial pathogens using standard agar diffusion methods, respectively. The minimum inhibitory concentrations (MIC) of the extracts were also determined using the microdilution method. The extract exerted significant and dose-related antidiarrhoeal activity when compared to the control and the standard drug, loperamide. The extract displayed highest antidiarrhoeal activity at a dose of 400 mg/kg which is comparable with that of the standard drug loperamide. The extract also showed moderate antibacterial effects against most of the test microorganisms except *S. typhimurium.* Phytochemical analysis of the extract revealed the presence of polyphenolic compounds such as flavonoids and tannins among others. The present study supports the folkloric use of the plant for the treatment of diarrhoeal diseases.

INTRODUCTION: Diarrhea is an alteration in the normal bowel movement, characterized by increased frequency of bowel sound and movement, wet stool, and abdominal pain. Clinically, it is used to describe increased liquidity of stool, usually associated with increased stool weight and frequency.

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Regardless of the understanding causes, treatment and prevention of diarrheal diseases, an estimated 4.6 million people, with 2.5 million children, die from diarrhea every year, particularly in developing countries.

Diarrhea may be acute or chronic. Acute diarrhea is the most common is usually caused by an infectious agent, even though drugs, poisons or acute inflammatory reactions can contribute a lot. Now a days, *rotavirus* is the major causative agent for infectious diarrhea, particularly in young children, however, other viral (*adenovirus, enterovirus, and norovirus*), bacterial (*Escherichia coli, Salmonella sp., Shigella sp., Campylobacter*

and *Vibrio cholerae*) and parasitic (*Cryptosporidium* and *Giardia*) agents are important pathogens [1-3].

Oral Rehydration Therapy (ORT) is considered life-saving in the management diarrhea especially in children, although it does not reduce the volume or duration of diarrhea. Likely, antibiotics and gut motility suppressing agents bid the other treatment option, wherein reverse dehydration, shorten the length of illness and reduce the period when an individual is infected. Treatment with pharmacological agents that are pathogen-specific or that suppress severe symptoms would be of benefit to patients suffering from prolonged diarrhea.

However, multidrug-resistant enteric pathogens, including *Shigella* spp, diarrheagenic *E. coli*, and *Salmonella* spp., are rapidly growing resistance to currently available antibiotics[1]. Therefore, there is an urgent need to discover and develop more effective and new antimicrobial agent. Herbal medicines are an alternative widely used for the treatment of diarrhea. They constitute an indispensable component of the traditional medicine practiced worldwide due to accessibility, ancestral experience, and economic viability. Despite the availability of a vast spectrum of pharmacological approaches for diarrheal management, the vast majority of people in developing countries rely on herbal drugs for its management.

The World Health Organization (WHO) has encouraged the study of the treatment and prevention of diarrheal diseases based on traditional medical practices [4]. Many animal-based studies have investigated the bioactivity and effects on the intestinal function of plants traditionally used as treatments for diarrhea.

Plant extracts can have antispasmodic effects, delay gastrointestinal transit, suppress gut motility, stimulate water adsorption or reduce electrolyte secretion [5]. *S. schimperi* is quoted by many traditional healers and ordinary people as a plant used as an antidiarrheal and antibacterial medicinal plant in Ethiopia. The aim of the present study was to evaluate the possible antidiarrhoeal (*in-vivo*) and antibacterial (*in-vitro*) properties of the hydroalcoholic extract of *S. schimperi*, to verify scientifically the claimed biological activities of the plant.

MATERIALS AND METHODS:

Collection and Drying of Leaves: Fresh leaves of *S. schimperi* were collected from North Shewa, Ethiopia in October 2011. The identity of the plant was confirmed at the National Herbarium, Department of Biology, Addis Ababa University where the voucher specimen was deposited. The leaves were air-dried at room temperature, and the dried leaves were then ground using sterile porcelain mortar and pestle.

Preparation of Extract: Extraction was carried out by soaking the dried and powdered plant material (200 g) in a flask (1.5 L) of 80% methanol and keeping for 72 h with occasional stirring. After 72 h, the extract was filtered using Whatman no. 1 filter paper. The extract was then concentrated by evaporating the methanol using a rotary evaporator under reduced procedure at 30 °C. The extract was then dried in a vacuum oven at 35°C.

Phytochemical Screening: Preliminary phytochemical screenings for the presence or absence of alkaloids, terpenoids, tannins, and flavonoids was chemically tested qualitatively using standard procedures [6].

Animals: Experiments were performed on 6- to 8-week-old Swiss albino mice of either sex (20–30 g) obtained from the animal house of Ethiopian Health and Nutrition Research Institute. They were housed in polyacrylic cages and fed with standard rodent pellet diet and given water *ad libitum*. All the animal experiments were performed by the rules and regulations approved by the institutional animal care and use committee.

Bacterial Strains: *Salmonella typhi, Salmonella paratyphi, Salmonella typhimurium, Shigella species, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli*, all clinical isolates were obtained from Akililu Lema Institute of Pathobiology, Microbiology Department, Addis Ababa University, Addis Ababa, Ethiopia.

Acute Oral Toxicity Test: In the acute oral toxicity study of 80% methanol extract of *S. schimperi*, a limit dose of each 2000 mg/kg body weight of the animal was administered on a single

test animal orally by gavage. The limit test was repeated three times on a single test animal as a part of an oral acute toxicity assay. As no mortality of experimental animals was observed at the limit dose for the LD_{50} study, a dose regime of more than the limit dose, *i.e.,* 2000 mg/ kg body weight was planned and performed on a single test animal at a time and repeated three times [7].

Castor Oil Induced Diarrhea: The antidiarrhoeal activity of the leaf hydroalcoholic extract of *S. schimperi* was studied using a castor oil-induced diarrhoeal test according to the method described by Amabeoku and Bamuamba [8]. Group I (received 1% tween 80 at a dose of 10 ml/kg) served as control group, Group II received the standard drug loperamide 3 mg/ kg, p.o. Group III, IV, and V received the methanol leaf extract of *S. schimperi* at the doses of 100, 200 and 400 mg/kg p.o., respectively. One hour after administration, all animals received 0.5 ml of castor oil, and then they were individually placed in cages the floor of which was lined with transparent paper. During an observation period of 4 h, the time of onset of diarrhea, the frequency of defecation and weight of feces excreted by the animals were recorded.

Antibacterial Activity Study: Agar well diffusion method was used to determine antibacterial activity. Diluted inoculums (0.1 mL) of test organism (10^6cfu/mL) were spread on Muller-Hinton agar plates. Wells of 8 mm diameter were punched into the agar medium with sterile cork borer under aseptic conditions and filled with 50 µl of 250 mg/ml of plant extract, solvent blank and standard antibiotic (gentamycin). The plate was kept at room temperature for 2 h for diffusion and was then incubated for 24 h at 37 °C. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms [9]. Gentamycin (0.1 mg) was used as a reference standard, and dimethylsulphoxide (DMSO) was used as a control. The growth was compared with the reference as well as the control.

Determination of Minimum Inhibitory Concentration (MIC): Microdilution broth method was used to determine MIC. The minimum concentration of hydroalcoholic extract of *S. schimperi* inhibiting growth of one or more the microorganisms was tested for MIC. Serial dilutions were prepared from 250 mg/ml of the extract using DMSO to make 250, 125, 62.5, 31.25, and 15.625 mg/ml solutions. The wells were inoculated with 0.1 mL aliquot of test microorganisms (10^6 CFU/mL) having serial dilutions of the extract (50 µl, each). The microplate was incubated at 37 °C ± 1 °C for 24 h. Dilution of the extract corresponding to respective test organism showing no visible growth was considered as MIC [10].

Statistical Analysis: The data are represented as mean ± SEM, and statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey post test where $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION:

Phytochemical Screening: The results of the phytochemical screenings on the hydroalcoholic extract of *S. schimperi* are shown in **Table 1**. The study showed the presence of flavonoids, tannins and terpenoids, and absence of alkaloids.

TABLE 1: PHYTOCHEMICAL SCREENING OF HYDROALCOHOLİC EXTRACT OF *SALVİA SCHİMPERİ* LEAVES

Secondary metabolite tested	Reagents used	Result
Alkaloids	Dragendorff's reagent Mayer's reagent	Absent
Flavonoids	Shinoda reagent Lead acetate	present
Tannins	Gelatine test	present
Terpenoids	Chloroform/H_2SO_4	present

Acute Toxicity Studies: At acute toxicity level, the extracts did not cause any mortality or visible signs of toxicity or differences in food and water uptake in the animals up to 2000 mg/kg. Thus, the extract is considered safe.

Antidiarrhoeal Effects: The result of the antidiarrhoeal activity of *S. schimperi* leaf extract screened against castor oil induced diarrhea in mice are presented in **Table 2**. The hydroalcoholic extract of *S. schimperi* leaves showed significant and dose-related antidiarrhoeal activity ($P<0.001$) against castor oil induced diarrhea in mice. The extract reduced the number of wet feces produced by castor oil administration from 8.2 ± 1.5 to 6.4 ± 0.9, 8.2 ± 1.5 to 2.2 ± 0.2 and 8.2 ± 1.5 to 1.6 ± 0.2 when experimental animals were respectively

administered 100, 200 and 400 mg/kg plant extract. The antidiarrhoeal effect of the extract at 400 mg/kg is comparable with the standard drug loperamide at 3 mg/kg.

TABLE 2: EFFECT OF HYDROALCOHOLIC EXTRACT OF *SALVIA SCHIMPERI* ON CASTOR OIL INDUCED DIARRHEA IN MICE

Groups	Dose (mg/kg, p.o.)	Time of onset of diarrhea (min.)	Total number of feces in 4 h	Weight of weight stool (g)
Group I	-	91 ± 10.9	8.2 ± 1.5	0.72 ± 0.07
Group II	3	237.8 ± 2.0*	1 ± 0.3*	0.04 ± 0.05*
Group III	100	135.2 ± 15.3	6.4 ± 0.9	0.36 ± 0.08**
Group IV	200	196 ± 14.5*	2.2 ± 0.2*	0.24 ± 0.05*
Group V	400	222.4 ± 11.3*	1.6 ± 0.2*	0.1 ± 0.04*

Significantly different when compared with that of the control at *P<0.001; **P<0.01 compared to control, results are mean ± SEM.

Due to the rapid development of resistance and high cost of the new generation antibiotics, lots of efforts are being made to discover new antimicrobial agents from different sources. Medicinal plants are considered useful and economically essential. The plant extracts have been developed and proposed for use as antimicrobial substances. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases.

Groups of phytochemical compounds commonly implicated for antimicrobial activity in medicinal plants are flavonoids, alkaloids, tannins, and triterpenoids. Presence of these bioactive components in the crude extracts could be linked to their activities against microorganisms. The demand for more and more drugs from plant sources is continuously increasing. It is therefore essential to evaluate plants of medicinal value systematically for various ailments that are used in traditional medicine. Hence, there is a need to screen medicinal plants for their promising biological activity [11, 12]. Thus, we evaluated the antibacterial and antidiarrhoeal activity of *S. schimperi*.

Ricinoleic acid, the active principle in castor oil caused changes in mucosal cell layer permeability, electrolyte transport, and intestinal peristalsis, leading to prostaglandin secretion, which results in an increase in the secretion of water and electrolytes into the small intestine. The gut wall contains prostaglandins E and F with prostaglandin synthetase activity mainly in the mucosa that cause intestinal cramps and diarrhea which might be due to the effect on intestinal smooth muscle and secretion. Moreover, prostaglandin contributes to the pathophysiological functions in gastrointestinal tract [13].

Investigation of the hydroalcoholic extract of *S. schimperi* at doses of 100, 200 and 400 mg/kg, reduced significantly the time of onset of diarrhea, the frequency of defecation and the wetness of fecal droppings in a dose-dependent way when compared with untreated Tween 80, thereby exhibited anti-diarrhoeal activity. At a dose of 400 mg/kg, *S. schimperi* seems to show an equivalent effect to that of 3 mg/kg of loperamide **Table 2**. Anti-diarrhea activity was found in plants possessing tannins, alkaloids, saponins, flavonoids, steroids, and terpenoids. Anti-diarrhea activities of flavonoids have been ascribed to their ability to inhibit intestinal motility and hydroelectrolytic secretions which are known to be altered in diarrhoeic conditions.

Tannins present in anti-diarrhea plants denature proteins in the intestinal mucosa by forming protein tannates which may reduce secretion. Studies on the functional role of tannins also reveal that they could also bring similar functions by reducing the intracellular Ca^{2+} inward current or by activation of the calcium pumping system (which induces the muscle relaxation[10]. Phytochemical screening of *S. schimperi* leaf extract revealed the presence of tannins, flavonoids, and terpenoids.

The anti-diarrhoeal activity may thus be attributed to the presence of tannins and flavonoids, the phytochemicals which are known to reduce the effect through denaturing the proteins by the formation of protein tannate, thereby causing the intestinal mucosa more resistant and reduces secretion [13].

Antibacterial Activity: Antibacterial activities of *S. schimperi* leaf extract screened against human pathogenic bacterial strains are presented in **Table 3**. The results of the antibacterial activity studies show the hydroalcoholic extract of *S. schimperi* possesses highest antibacterial activity (16 mm) against *P. aeuroginosa* and the lowest activity against *S. typhi and S. paratyphi* (10 mm & 11 mm, respectively). The extract did not show any inhibition against *S. typhimurium*. However, the extract showed a good effect against *Shigella* sp against on which the standard antibiotic did not affect.

TABLE 3: ANTIBACTERIAL ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT OF *SALVIA SCHMPERI* AND GENTAMYCIN

Organisms	Inhibition zones (mm)	
	Extract	Gentamycin
S. typhi	10	18
S. paratyphi	11	16
S. typhimurium	NE	16
Shigella spps	14	0
P. aeuroginosa	16	19
S. aureus	14	20
E. coli	14	20

MIC of the Extract of *S. schimperi* Leaves: The methanol extract of *S. schimperi* leaves showed good antibacterial activity against different enteric pathogens. Hence, we determined the MIC of methanol extract against different enteric pathogens. MIC value indicates the lowest concentration of the extract that exhibits the growth inhibition of particular bacteria. MIC value of the methanol extract of *S. schimperi* leaf was presented in **Table 4**.

TABLE 4: MINIMUM INHIBITORY CONCENTRATIONS OF HYDROALCOHOLIC EXTRACT OF *SALVIA SCHIMPERI*

Organisms	Minimum inhibitory concentrations (mg/mL)
S. typhi	31.25
S. paratyphi	62.5
S. typhimurium	NE
Shigella spps	62.5
P. aeuroginosa	125
S. aureus	31.25
E. coli	125

In this study, we have shown the antibacterial activity of *S. schimperi* on a wide range of enteric pathogens which can cause severe diarrhea diseases and dysentery. We have also identified the phytochemical constituents which are known to have antimicrobial and antidiarrhoeal activity.

CONCLUSION: To conclude, the present investigation reveals the antibacterial nature of the plant and suggests that the plant could be exploited in the management of diseases caused by the tested bacteria in human. Furthermore, the hydroalcoholic extract of *S. schimperi* in graded doses reduced diarrhea and thereby lending support to use *S. schimperi* in folklore medicine against chronic diarrhea. The phytochemical analysis revealed that flavonoids and tannins present in the methanolic extract of *S. schimperi* are thus responsible for the antibacterial and antidiarrhoeal activity.

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CONFLICT OF INTEREST: Nil

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