ORIGINAL ARTICLE

Discovery Phytomedicine 2016, Volume 3, Number 4: 22-28

In vivo antidiarrheal activity evaluation of the seeds of *Sorghum bicolor L.* (Poaceae)

Zelalem Hunegnaw,[1] Dessalegn Asmelashe Gelayee,[*2] Zewdneh Shewamene Sabe[3]

ABSTRACT

The seeds of *Sorghum bicolor* (Family: Poaceae) have been used traditionally for treatment of diarrhea but its efficacy needs to be evaluated. This study was carried out to evaluate the *in vivo* antidiarrheal activity of 80% methanol crude extract of the seeds of locally grown *S. bicolor.* This screening was conducted by using three standard methods of bioassay: castor oil-induced diarrhea, castor oil-induced enteropooling and castor oil-induced gastrointestinal transit tests. Mice were randomly divided in to 6 mice per group of five groups. The negative control group received 1% tween 80, the positive control received loperamide 5 mg/kg and the test groups received orally three different doses of the 80% methanolic seed extract of *S. bicolor* (100, 200 and 400 mg/kg body weight). Depending on the model, data on the onset of defecation, number of wet feces, total number of feces, weight of intestinal fluid as well as length of intestinal transit were collected. In addition, preliminary phytochemical and acute toxicity studies were conducted. The presence of phenols, flavonoids, tannins, terpenoids and sterols in the extract was confirmed. On the other hand, the oral median lethal dose (LD_{50}) of the extract was estimated to be higher than 2000 mg/kg. The 200 mg/kg and 400 mg/kg extract doses especially demonstrated a very significant ($P < 0.01$) inhibitory activity against castor oil-induced diarrhea, castor oil-induced enteropooling as well as castor oil-induced gastrointestinal transit. This study therefore demonstrated the presence of pharmacologic activity against diarrhea by the crude extract of *S. bicolor* and validates its antidiarrheal use in traditional medicine system. Mechanisms for antidiarrheal activity may be partly inhibition of motility as well as secretion of the gastrointestinal tract. Therefore, we suggest further studies on *Sorghum bicolor* in the search for better alternatives to the contemporary antidiarrheal drugs.

Key words: antidiarrheal, extract, *in vivo, Sorghum bicolor*

*Corresponding Author: Dessalegn Asmelashe Gelayee, Department of Pharmacology, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia, E-mail: desefikir@gmail.com

Cite this Article: Hunegnaw Z., Gelayee D.A., Sabe Z.S. 2016. *In vivo* antidiarrheal activity evaluation of the seeds of *Sorghum bicolor L.* (Poaceae) Discovery Phytomedicine 3(4): 22-28. DOI:10.15562/phytomedicine.2016.32

INTRODUCTION

Diarrhea is characterized by an increase in the frequency of bowel movements, abdominal pain, and bowel discharge of semisolid or watery fecal matter (three or more times in a day).[1] It is mainly caused by imbalances in several pathways (eg acetylcholine, dopamine, and catecholamine) as well as infectious agents, plant toxins, and inflammatory problems.[2] The disease is known to be a major cause of morbidity and mortality worldwide with approximately 1.87 million deaths in children under 5 years particularly in developing countries.[3]

Many people in the developing countries still rely on traditional healing practices involving medicinal plants for their daily health care needs. In Africa, medicinal plants have been commonly used for treatment of several diseases including diarrhea. It has been frequently described that more than 80% of the population have been using traditional medicine.[4] Therefore, the World Health Organization encouraged studies for the treatment and prevention of diarrheal diseases depending on traditional medical practices.[5]

Sorghum bicolor L (Family: Poaceae), locally known as zengada, is an annual plant which constitutes a major food crop in Africa, Europe, Asia, and America. It serves as a major source of proteins, calories, mineral and is the fifth most important cereal crop in the world.[6] The seeds have been used for management of diarrhea.[7, 8] It is also a folk remedy for cancer, epilepsy, stomach ache, and malaria.[9]

Several studies have shown that *S. bicolor* has antioxidant, antiinflammatory, anticancer and cholesterol-lowering effects and can reduce the risk of cardiovascular diseases. In addition, it possesses good antimicrobial activity against *S. auerus* and *E.coli*.[10, 11]

However, there is limited scientific evidence supporting the potential use of *S. bicolor* as an antidiarrheal agent. The above scientific studies and ethnobotanic claims about the plant as well as the limited data available to validate the antidiarrheal acivity have brought this work into reality. Moreover current drugs used in management of diarrhea are characterized by several limitations such as adverse

[1]Department of Pharmacology, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia; [2]Department of Pharmacology, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia; [3]Department of Pharmacology, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia.

effects, contraindications as well as antimicrobial drug resistance and hence it is very important to look for a novel antidiarrheal drug with improved profiles. Therefore, the present study was conducted to evaluate the acute toxicity and *in vivo* antidiarrheal activity of the seeds of *Sorghum bicolor* in experimentally induced acute diarrhea in mice.

RESEARCH DESIGN AND METHODS

Plant collection and Preparation of the Extract

The seeds of *Sorghum bicolor* were collected in January 2015 from farms around Gondar town of Northwest Ethiopia. This was then identified and authenticated at the National Herbarium, Addis Ababa University, Addis Ababa, Ethiopia where a voucher specimen (*Sorghum bicolor l.* 03) was deposited. The seeds were cleaned of extraneous matter, air-dried under shade at room temperature for 6 weeks and then coarsely powdered using mortar and pestle. A weighed amount of the powder was macerated using 80% methanol for 72 hr with occasional stirring. The resultant mixture was filtered with Whatman filter paper (Grade 1) and the residue was further re-macerated twice. Finally the filtrates were combined together, concentrated by oven (Leaders Engineering, UK) at 40°C and the dried extract was stored at 4°C using air-tight container until further use. The extract was reconstituted in 1% Tween 80 at appropriate concentrations for the various experiments conducted.

Experimental animals

Adult Swiss albino mice of either sex weighing between 20-25 g were obtained from animal house of Pharmacology Department, University of Gondar. The animals were housed in a standard polyethylene glycol cage. They were fed with standard diet and water *ad libitum*, and allowed a one-week acclimatization period prior to the study. The animals were maintained under standard conditions of humidity, temperature and 12 h light/12 h darkness cycle. The mice that were used for the antidiarrheal test were only those that developed diarrheal response with castor oil at doses employed for the main tests.

Phytochemical Screening

The crude hyroacoholic extract of *Sorghum bicolor* was subjected to qualitative phytochemical screening according to standard methods.[12, 13]

TEST FOR ALKALOIDS

Thoroughly ground material (2 g) was treated in a test tube with 10 ml of 1% HCl for 30 min in a water bath. The suspension was filtered through cotton into test tube and was divided into two parts and to one part of the solution five drops of Dragendorff's reagent and to the other part five drops of Mayer's reagent were added. If the alkaloids are present, the test with Dragendorff's reagent should form a yellowish orange precipitate or a whitish opalescence with Mayer's reagent.

TEST FOR SAPONINS

An aliquot of the extract in a 15 ml test tube was vigorously shaken for 2 min. The frothing which persists for 15 min was inspected for indication of the presence of saponins in the extracts.

TEST FOR PHENOLS

A mixture of one ml 1% $FeCl_3$ and one ml of 1% $K_3Fe(CN)_6$ was prepared immediately before this test. Then, to two ml of filtered solution of the aqueous macerate of plant material, three drops of a mixture of 1% $FeCl_3$ and 1% $K_3Fe(CN)_6$ were added. The final solution should form a green blue colour if it contains phenolic compounds.

TEST FOR FLAVONOIDS

The dried 80% methanolic extract (100 mg) was dissolved in a mixture of methanol and water. To 2 ml of the extract solution, three to five drops of 2% lead acetate solution were added. Then, it was observed whether it develops yellow or orange colour which indicates the presence of flavonoids.

TEST FOR TANNINS

The 80% methanol extract (3 g) was heated in a test tube with 10 ml of distilled water on a water bath for five minutes. After cooling, the solution was filtered through filter paper and 5 ml of 2% sodium chloride was added to the clear filtrate. The suspension was filtered (Whatman No. 1) and five ml of 1% gelatin was added to the clear filtrate. Then, the filtrate was observed whether it gives a precipitate which disappears upon addition of excess gelatin solution indicating the presence of tannins.

TEST FOR TERPENOIDS

Five milliliter of plant extract was mixed in 2 ml of chloroform followed by the careful addition of 3 ml concentrated (H_2SO_4). A layer of the eddish brown colouration formed at the interface indicates a positive result for the presence of terpenoids.

TEST FOR STEROIDS

The concentrated residue was dissolved in chloroform and treated with 3 drops of solutions of cold mixture of concentrated sulphuric acid and 1 ml of acetic anhydride. The formation of rose color (reddish brown) that changes to greenish blue indicates the presence of steroidal compounds.

Preliminary Acute Toxicity Test

The acute toxicity median lethal dose (LD_{50}) of the extract was estimated p.o. in Swiss albino mice following the 2008 Organization for Economic Co-operation and Development (OECD) guideline.[14]

Accordingly, a limit test was done at 2000 mg/kg using a total of three mice with each mouse given the extract sequentially in 48 hr. The mice were carefully observed for a total of 15 days for any change in body weight (weekly), changes in skin and fur, lacrimation, salivation, diarrhea, lethargy, sleep, coma and death.

Grouping and Dosing of Animals

Animals were divided into a negative control, a positive control and three test groups of six animals each. The negative control received 1% tween 80 and the positive control received loperamide (5 mg/kg) and the test groups received orally different doses of the 80% methanolic seed extract of *S. bicolor* (100, 200 and 400 mg/kg body weight). Doses were determined based on the acute toxicity studies as per OECD guidelines 425.[14] After having considered the safety of the plant, 1/10th of the maximum dose (2000 mg/kg) was considered as a middle dose. Half and double of the middle dose was selected and considered as low and high doses, respectively

Castor oil-induced diarrhea

This test was conducted according to the method described by Awounters et al with some modifications.[15] Thirty albino mice of either sex weighing 20-25 g were divided in to five groups of six animals each. They were fasted for 18 hours before commencing the experiment but allowed free access to water. Group 1 were treated with 1% tween 80 (10 ml/kg body weight) and Group 2 with loperamide (5 mg/kg) via orally. The 3rd, 4th, and 5th groups respectively were treated with 100 mg/kg, 200 mg/kg and 400 mg/kg doses of the crude 80% methanolic extract of *S. bicolor.* After 30 minutes, the animals of each group were treated with 1 ml of castor oil orally. The animals were then placed on individual cages over clean Whatman filter paper which was changed every hour. Diarrhoea (wet, unformed stool) was assessed for 4 hrs and the result was expressed using the following formula.

$$\%\text{Inhibition of diarrhea} = (Nc - Nt) / Nc \times 100$$

Where, Nc = Mean number of wet defecation in control group, Nt = Mean number of wet defecation in test group

Castor oil-induced enteropooling

This test was conducted according to the method described by Robert et al with some modifications.[16] Thirty albino mice of either sex weighing 20-25 g were divided in to five groups of six animals each. They were fasted for 18 hours before commencing the experiment but allowed free access to water. Group 1 were treated with 1% tween 80 (10 ml/kg body weight) and Group 2 with loperamide (5 mg/kg) via orally. The 3rd, 4th, and 5th groups respectively were treated with 100 mg/kg, 200 mg/kg and 400 mg/kg doses of the crude 80% methanolic extract of *S. bicolor.* After 30 minutes, the animals of each group were treated with 1 ml of castor oil orally and then thirty minutes later each mouse was sacrificed and the whole length of the intestine from pylorus to caecum was removed immediately and intestinal fluid was collected into pre-weighed test tubes for weight determination. The percentage inhibition of fluid accumulation was calculated as follows.

$$\%\text{ Inhibition of diarrhea} = (Wc - Wt)/ Wc \times 100$$

Where: Wc = Mean weight of intestinal fluid in control group Wt = Mean weight of intestinal fluid in the test group

Castor oil- induced intestinal transit in mice

This test was conducted according to the method described by Mascolo et al.[17] with some modifications. Thirty albino mice of either sex weighing 20-25 g were divided in to five groups of six animals each. They were fasted for 24 hrs before commencing the experiment but allowed free access to water. Group 1 were treated with 1% tween 80 (10 ml/kg body weight) and Group 2 with loperamide (5 mg/kg) via orally. The 3rd, 4th, and 5th groups respectively were treated with 100 mg/kg, 200 mg/kg and 400 mg/kg doses of the crude 80% methanolic extract of *S. bicolor* 1 hour before the administration of castor oil. Then 1 ml of charcoal meal (3% deactivated charcoal in normal saline) was administered orally 1 hour after the castor oil treatment. Then each mouse was sacrificed 1 hour later with an ether anesthesia and the small intestine was immediately isolated. The percentage distance of the small intestine, from the pylorus to the caecum, travelled by the charcoal plug was determined based on the distance travelled by charcoal in reference to total intestinal length. The percentage inhibition of intestinal transit was calculated as follows.

$$\%\text{Inhibition of intestinal transit} = (\%Tc - \%Tt)/ \%Tc \times 100$$

Where: %Tc = Mean percent of intestinal distance travelled by charcoal in control group, %Tt = Mean percent of intestinal distance travelled by charcoal in test group.

Statistical analysis

Results were expressed as mean + SEM. The significance of differences between the control and treated groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. P value less than 0.05 were considered statistically significant.

Ethical clearance

The animals were handled according to the international animal care and welfare guideline[18] and an ethical clearance was taken from research and ethics review committee of Department of Pharmacology, University of Gondar.

RESULTS

The 80% methanolic crude extract of the seeds of *Sorghum bicolor* was dark red, hygroscopic semi-solid at room temperature with a percentage yield of 1.67% (w/w).

Phytochemical screening

Phytochemical analysis of the crude extract gave positive reaction for each of the following secondary metabolites: phenols, flavonoids, tannins, terpenoids, and steroids. (Table 1)

Acute toxicity test

There was no any mortality, behavioral, neurological or physical changes characterized by symptoms such as reduced motor activity, restlessness, convulsions, coma, diarrhea and lacrimation at the limit dose of 2000 mg/kg of the crude extract during the two weeks observation period. This signifed that the LD_{50} of the 80% methanolic crude extract of seeds of *S. bicolor* is greater than 2000 mg/kg.

Effect of S. bicolor *against castor oil-induced diarrhea*

When compared to the negative control, the crude extract administered orally exhibited very significant anti-diarrheal effect against castor oil induced diarrhea in the mice as shown in Table 2. The delay in onset of diarrhea as well as the decrease in number of wet feces as well as both dry and wet faces induced by extract treatment at all test doses were very significant ($P < 0.01$). The percent diarrheal inhibition was highest (68.55%) for the 400 mg/kg extract dose. The standard drug, loperamide (5 mg/kg), also significantly ($P < 0.01$) inhibited diarrhea in all the above parameters. (Figure 1)

Effect of S. bicolor *against castor oil induced enteropooling*

Treatment with the extract significantly decreased intestinal fluid weight (g) at all test doses but it was very significant ($P < 0.01$) with the two higher doses. The percentage reductions of castor oil induced enteropooling by the extract was dose dependent and it was highest (39.77%) for the 400 mg/kg dose while that of loperamide (5 mg/kg) was 64.77%. (Table 3, Figure 2)

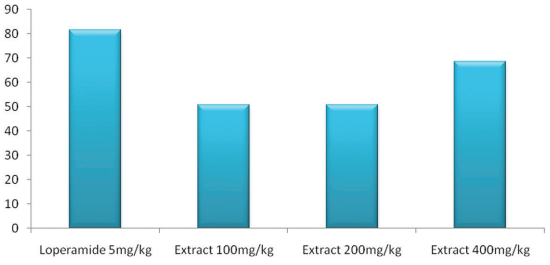

Figure 1 Percentage inhibition against castor oil induced diarrhea by methanolic extract of seeds of *S. bicolor L.* (*P < 0.01 for all treatment groups*)

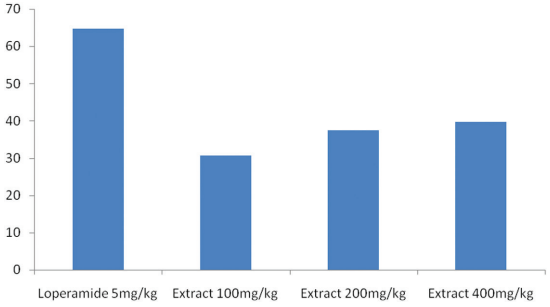

Figure 2 Percentage inhibition against castor oil induced enteropooling by methanolic extract of seeds of *S. bicolor L.*

Table 1 **Results of phytochemical screening of methanolic seed extract of *Sorghum bicolor L***

Serial number	Tests	Extract
1	phenols	+
2	Saponins	_
3	Alkaloids	_
4	Flavonoids	+
5	Tannins	+
6	Terpenoids	+
7	Sterols	+

(+) = Present, (-) = Absent

Table 2 **Effect of *S. bicolor* extract against castor oil induced diarrhea**

Treatment	Onset of diarrhea (minutes)	Number of wet feces	number of wet and dry feces	Percent of inhibition
1% Tween 80 10 mg/kg	26.00±0.52	10.83±0.31	13.5±0.5	-
Loperamide 5 mg/kg	57.00±0.82**	2.00±0.00**	5.33±0.21**	81.53
S. bicolor 100 mg/kg	36.83±0.31**	5.33±0.21**	8.33±0.21**	50.78
S. bicolor 200 mg/kg	42.5±0.43 **	5.33±0.21**	7.83±0.31**	50.78
S. bicolor 400 mg/kg	45±3.64**	4.50±0.22**	7.33±0.33**	68.55

*Values are expressed as mean ± SEM (n=6); ** = P <0.01, Very significant compared with –ve control; * = P <0.05, Significant compared with –ve control*

Table 3 **Effect of *S. bicolor* extract against castor oil induced enteropooling**

Treatment	Weight of intestinal fluid (g)	Percent inhibition
1% Tween 80 10 mg/kg	0.88±0.09	-
Loperamide 5 mg/kg	0.31±0.02**	64.77
S. bicolor 100 mg/kg	0.61±0.08*	30.68
S. bicolor 200 mg/kg	0.55±0.035**	37.5
S. bicolor 400 mg/kg	0.53±0.02**	39.77

*Values are expressed as mean ± SEM (n=6); ** = P <0.01, Very significant compared with –ve control; * = P <0.05, Significant compared with –ve control,*

Table 4 **Effect of *S. bicolor* extract on intestinal transit of charcoal meal in mice**

Treatment	% traverse by charcoal meal	Percent inhibition
1% Tween 80 10 mg/kg	47.5±3.71	-
Loperamide 5 mg/kg	20.83±2.45**	56.15
S. bicolor 100 mg/kg	34.5±3.2ns	27.36
S. bicolor 200 mg/kg	28.66±2.46**	39.66
S. bicolor 400 mg/kg	27.75±1.73**	41.58

*Values are expressed as mean ± SEM (n=6); ** = P<0.01, Very significant compared with –ve control; ns = P >0.05, Non significant compared with –ve control*

Effect of S. bicolor *on castor oil induced gastrointestinal motility*

The extract demonstrated a dose dependent slowing of the propulsion of charcoal meal through the gastrointestinal tract but only the 200 mg/kg and 400 mg/kg extract doses did so very significantly ($P < 0.01$) when compare to the control group as shown in Table 4 and Figure 3.

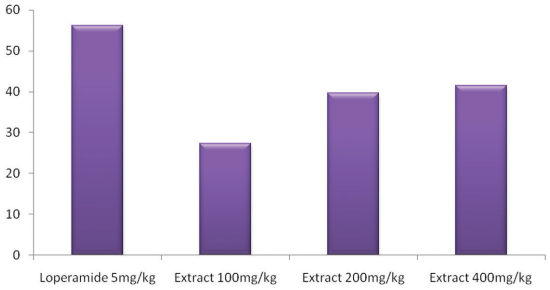

Figure 3 Percentage inhibition against gastrointestinal motility by methanolic extract of seeds of *S. bicolor L.*

DISCUSSION

This study was carried out to evaluate the antidiarrheal activity of the seeds of *Sorghum bicolor* in experimentally induced acute diarrhea in mice. The seeds of *sorghum bicolor* have been used for treating diahrrea by the local community despite the absence of any scientific validation for its safety and efficacy.[7,8] However, it is evidenced to have antimicrobial activity against *S.auerus* and *E. coli.*[10] The 80% methanolic solvent employed in this study was based on its good ability to extract a wide variety of polar and moderately polar compounds.[19]

Diarrhea is usually considered a result of altered motility and fluid accumulation within the intestinal tract and thus many antidiarrheal agents act by reducing the gastrointestinal motility and / or the secretions.[20] In relation to this, the antidiarrheal activities of several plants have been validated by screening their activity on the gastrointestinal motility and its secretions.[21]

The induction of diarrhea by castor oil is recommended for study of the anti-secretory and antimotility potential of medicinal plants.[1]

The present study demonstrated that *S. bicolor, especially at the 200 mg/kg and 400 mg/kg doses,* had very significant antidiarrheal activity in castor oil induced diarrhea, enteropooling as well as intestinal transit models validating the antidiarheal traditional use of the plant. Castor oil or its active principle recinolic acid induces diarrhea because of increased peristaltic activity and changes in electrolyte permeability of the intestinal membrane through elevated prostaglandin biosynthesis and release. It decreases fluid absorption, increases secretion in the small intestine and colon. In addition inhibitors of prostaglandin biosynthesis delay castor oilinduced diarrhea.[15] Thus the results in the present study suggest that *S. bicolor* extract may possess antisecretory activity. It may also possess *in vivo* spasmolytic activity and increased the reabsorption of sodium chloride and water by

decreasing intestinal motility as observed by the decrease in intestinal transit by charcoal meal.

The highest antidiarrheal activity of the largest dose of the extract in the present study is in line with similar studies on other plants.[22]

The standard drug, Loperamide, demonstrated the highest and very significant ($p < 0.01$) antidiarrheal effects in all parameters used in the castor oil models. This drug exerts its antidiarrheal activity by mechanisms including slowing down intestinal motility and anti secretory properties.[23] Earlier studies have reported that antimotility and antidiarrheal properties of medicinal plants are due to tannins, alkaloids, saponins, and sterols.[24]

The antidiarrheal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretions.[25, 26]

Tannins denature proteins in the intestinal mucosa by forming protein tannates which make intestinal mucosa more resistant to chemical alteration and reduce secretion.[27]

Therefore the antidiarrheal activity of *S. bicolor* in the present study might arise from phenols, flavonoids, tannins, terpenoids and sterols alone or in combination. Moreover, the acute toxicity study revealed the safety of the extract at the limit dose of 2000 mg/kg in mice via oral ingestion. Therefore the LD_{50} value of the extract of the plant is determined to be above 2000 mg/kg which indicates that the seeds of the plant can be safely ingested orally.

CONCLUSION

This study demonstrated that the seeds of *Sorghum bicolor* contains pharmacologically active substances effective for management of diarrhea. Thus it validated the antidiarrheal use of the seeds in the traditional medicine system. Inhibition of motility and secretion of the gastrointestinal tract may partly be the mechanisms by which the extract is acting. Therefore, the seeds of this plant are a potential candidate to answer the quest for novel antidiarrheal agents. We suggest further investigation for isolation, identification and characterization of different active compounds from the seeds and for elucidating their mode of antidiarrheal action.

ACKNOWLEDGMENT

We express our appreciation to Mr Assefa Belay for his technical support.

CONFLICT OF INTEREST

We declare there is no conflict of interest.

AUTHORS CONTRIBUTION

ZH and DAG developed the proposal and ZH, DAG and ZSS finalized it. Experiment was carried by ZH, DAG, ZSS. Data analysis, manuscript development and final approval was done by ZH, DAG and ZSS.

FINANCIAL SUPPORT

This study was carried out by the financial support from University of Gondar.

REFERENCES

1. de Sales IR, Machado F, Marinho A, Lúcio A, Filho J, Batista L. Cissampelos sympodialis Eichl. (Menispermaceae), a medicinal plant, presents antimotility and antidiarrheal activity *in vivo* BMC Complementary and Alternative Medicine (2015) 15:253. http://dx.doi.org/10.1186/s12906-015-0578-7
2. Susan EA, Mays A. Pharmacology. In: The Merck veterinary manual. USA: Merck and Co. Inc; 2005
3. Boschi-Pinto C, Velebit L, Shibuya K. Estimating child mortality due to diarrhoea in developing countries. Bulletin of the World Health Organization. 2008;86:9.710-717. http://dx.doi.org/10.2471/blt.07.050054
4. Kassaye KD, Amberbir A, Getachew B, Mussema Y. A historical overview of traditional medicine practices and policy in Ethiopia. Ethiopian Journal of Health Development. 2006;20(2).127-134. http://dx.doi.org/10.4314/ejhd.v20i2.10023
5. Atta AH, Mouneir SM. Antidiarrheal activity of some Egyptian medicinal plant extracts. Journal of Ethnopharmacology. 2004; 92: 303-09. http://dx.doi.org/10.1016/j.jep.2004.03.017
6. Sule O, Yahaya A. Evaluation of the effect of sorghum bicolor aqueous extract on the haematological, renal and hepatic parameters in rats fed with low and high iron diet. European Journal of Medicinal Plants.2014;4(7):783-793. http://dx.doi.org/10.9734/ejmp/2014/7847
7. Giday M. An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia. Skriftserie. 2001;3:81-99.
8. Abate G. Etse Debdabe (Ethiopian Traditional medicine). Addis Ababa University Addis Ababa University press, Biology Department, Science Faculty;1989.
9. Joseph M, Lloyd W. Sorghum phytochemicals and their potential impact on human health. Phytochemistry. 2004;65 (9):1199-1221. http://dx.doi.org/10.1016/j.phytochem.2004.04.001
10. Hyun YK, Eun SS, Bimal KG, Ill-Min C, Soon S K, Eun J G, et al. Antioxidant and antimicrobial activities of crude sorghum extract. Food Chemistry. 2009;115(4).1234-1239. http://dx.doi.org/10.1016/j.foodchem.2009.01.032
11. Burdette A, Garner P, Mayer E, Hargrove J, Hartle D, Greenspan P. Anti-Inflammatory Activity of Select Sorghum (Sorghum bicolor) Brans. Journal of Medicinal Food. 2010;13(4):1-9. http://dx.doi.org/10.1089/jmf.2009.0147
12. Trease GE, Evans WC: A Textbook of Pharmacognocy. London: Bailliere Tindall Ltd; 1989.
13. Jones WP, Kinghorn AD. Extraction of Plant Secondary Metabolites. Methods in Molecular Biology.2012;864:341-66.
14. Organization for Economic Cooperation and Development: OECD Guidelines for the Testing of Chemicals. Acute Oral Toxicity -Up-And-Down-Procedure. UDP;425.France: OECD Publishing; 2008. http://dx.doi.org/10.1787/9789264071049-en
15. Awounters F, Niemegeers CJ, Lenaerts FM, Janseen PA. Delay of castor oil diarhoea in rats; a new way to

evaluate inhibitors of prostaglandin biosynthesis. Journal of Pharmacy Pharmacology. 1978;30:41-45. http://dx.doi.org/10.1111/j.2042-7158.1978.tb13150.x
16. Robert A., Nezamis, J.E., Lancaster, C., Hanchar, A.J., Klepper, M.S., Enteropooling assay; a test for diarrhea produced by prostaglandins.Prostaglandins.1976;11:809-828. http://dx.doi.org/10.1016/0090-6980(76)90189-1
17. Mascolo, N, Izzo, AA, Avotore, G, Barboto, F, Capasso, F. Nitric oxide and castor oil included diarrhea, Journal of Pharmacology and Experimental therapeutics.1994;268: 291-292. http://dx.doi.org/10.1111/j.1476-5381.1993.tb13478.x
18. Institute for Laboratory Animal Research (ILAR). Guide for the Care and Use of Laboratory Animals. 1996. National Academy Press. Washington, D.C.
19. Otsuka H. Purification by Solvent Extraction Using Partition Coefficient. Sarker D, Latif Z, Gray A, editors. Methods in Biothechnology Natural Products Isolation. New Jersey: Human Press;2006.p.269-273.
20. Akuodor GC, Muazzam I, Usman-Idris M, Megwas UA, Akpan JL, Chilaka KC, et al. Evaluation of antidiarrheal activity of methanol leaf extract of Bombax buonopozense in rats. Ibnosina Journal of Medicine and Biomedical Sciences. 2011;3:15-20.
21. Gutiérrez SP, Mendoz DZ, Munive AH, Martínez AM, González CP, Mendoza ES: Antidiarrheal activity of 19-deoxyicetexone isolated from Salvia ballotiflora Benth in mice and rats. Molecules. 2013;18:8895-8905. http://dx.doi.org/10.3390/molecules18088895
22. Tamiru W, Ejigu A, Endale A, Fikru A. Experimental assessment of antidiarrheal and antisecretory activity of 80% methanolic leaf extract of Zehneria scabra in mice. BMC Complementary and Alternative Medicine. 2014;14:460. http://dx.doi.org/10.1186/1472-6882-14-460
23. Ruppin H. Review: loperawide-a potent antidiarrhoeal drug with actions along the alimentary tract. Alimentary pharmacology & therapeutics. 2007;1(3):179-90. https://doi.org/10.1111/j.1365-2036.1987.tb00617.x
24. Kota BP, Teoh AW, Roufogalis BD. Pharmacology of traditional herbal medicines and their active principles used in the treatment of peptic ulcer, diarrhoea and inflammatory bowel disease. New Advances in the Basic and Clinical Gastroenterology. 2012;14:297-310. https://doi.org/10.5772/34107
25. Di Carlo G, Autore G, Izzo AA, Maibline P, Mascolo N,Viola P, Diurno MV, Capasso F. Inhibition of intestinal motility and secretion by flavonoids in mice and rats: Structure activity relationships. Journal of Pharmacy and Pharmacology. 1993;45:1054-1059. https://doi.org/10.1111/j.2042-7158.1993.tb07180.x
26. Rao VSN, Santos FA, Sobreika TT, Souza MF, MeloLL, Silveira ER. Investigation on thegastroprotective and antidiarrheal properties ofternatin,a tetramethoxyflavone from Egletes viscose. Planta Medica. 1997;63: 1496-1497. https://doi.org/10.1055/s-2006-957632
27. Havagiray R, Ramesh C, Sadhna K. Study of antidiarrhoeal activity of Calotropis gigantea R.B.R. in experimental animals. Journal of Pharmacy & Pharmaceutical Sciences. 2004;7:70-75.