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Evaluation of Antidiarrheal Activity of the Ethanolic Stem Bark Extract of Vernonia amygdalina in Experimental Animals

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Abstract
Locally, *Vernonia amygdalina* has been recommended for treatment of a number of ailments including stomach ache, skin infections, diabetes, insomnia, toothache, pneumonia, stroke and diarrhea. Despite its traditional usage as an anti-diarrheal agent, there is limited documented information on its effectiveness for the treatment of diarrhea. In this study, the anti-diarrheal activity of the ethanol extract of the stem bark of *Vernonia amygdalina* was evaluated in castor oil induced diarrheal rats. The anti-diarrheal effect of the extract at 100, 200 and 400mg/kg body weight was evaluated in female rats using gastro intestinal transit, diarrheal and enteropooling induced by castor oil models. Loperamide and Atropine sulphate were used as standard drugs for diarrheal. In castor oil induced diarrheal model, the ethanol stem bark extract of *Vernonia amygdalina* at 100, 200 and 400mg/kg and the standard drug Loperamide (3mg/kg) significantly reduced the time of onset of diarrhea and the frequency of defecation (total number faecal output and weight of faeces). The extract (100, 200 and 400mg/kg) also significantly reduced the intestinal transit time in charcoal meal and castor oil induced enteropooling when compared with Atropine sulphate (5mg/kg). The result showed that the ethanol extract of *V. amygdalina* stem bark have significant anti-diarrheal activity which support the traditional use of the plant for the treatment of diarrhea.
Keywords：*Vernonia amygdalina,* antidiarrheal, castor oil, ethanol extract.

Introduction

Diarrhea is defined as an increase in the number of stools, an increase in the fluidity of stools and or presence of blood and mucus with increase neutrophil polymorphos in the stool (Mathan, 1998) It results from alteration in normal bowel movement and it is characterized by an increase in the water content, volume or frequency of stools (Guerrant *et al*., 2001). The major causative agents of diarrhea in humans are *Shigella flexner, Staphylococcus aureus, Eschericha coli* and *Salmonella typhi* (Toyin *et al*., 2012). *Candida albicans* has also been known to cause diarrhea in humans (Robert *et al.*, 2001). Diarrhea is a major health problem worldwide especially in developing countries, it accounts for more than 5-8 millions deaths in infants and children under 5 years of age, every year (Shoba and Thomas, 2001). Many Government and International Organizations are trying to curb this disease but the rate of incidence is still high hence the need for continuous efforts for both treatment and prevention. Medicinal plants are a promising source of anti-diarrheal drugs , WHO has encouraged studies for treatment and prevention of diarrheal diseases using traditional medical practices (Atta and Mouneir, 2004). It is therefore important to identify and evaluate available natural drugs as alternative to currently used anti-diarrheal drugs. A range of medicinal plants with anti-diarrheal properties are widely used by traditional healers. However, the effectiveness of many of these anti-diarrheal plants has not been scientifically evaluated hence a justification for this study. *Vernonia amygdalina* commonly called bitter leaf is the most widely cultivated species of the genus *Vernonia* which has about 1000 species of shrub . It belongs to the family asteraceae. It is vegetatively cultivated by stem cutting and popularly distributed in most of West Africa countries. It was named after an English botanist William Vernon. It is also referred to as iron weed and frequently found in gardens . The leaves are characteristically bitter, although the bitterness can be abated by boiling or soaking in several changes of clean water. The stem and root of the bark are used as chew-sticks in Nigeria. More importantly, the leaves are used to prepare the very popular bitter leaf soup in Nigeria and are also reportedly consumed by goats in some parts of Nigeria (Aregheore *et al.*, 1998). *V. amygdalina* is a medicinal plant used in folk medicine to manage several ailments (Allen, 1987). The roots, stem and leaves are used in ethnomedicine to treat fever, kidney problem and stomach discomfort among several other uses (Burkhill, 1985; Chall and Willcox, 2009). Both aqueous and alcoholic stem, root and leaf extracts are reported to be extensively used as purgative and antimalarial as well as in the treatment of skin infections such as eczema, ringworms by applying to the affected part. Pharmacological studies have shown that the leaf extract has both hypoglycemic and hypolipidemic properties in experimental animals and so can be used in the management of diabetes mellitus (Akah and Okafor,1992). Inspite of the usefulness of various parts of the plant as a remedy against diseases and its nutritional relevance, literature have concentrated on the leaves whereas both root and stem barks have been

shown to be medically responsive. The root and stem barks are used in the management of diarrhea in folk medicine (Swee, 2010). However, the effectiveness of their anti-diarrheal activity has not been scientifically evaluated. Hence the study was undertaken to evaluate the ethanolic stem bark extract of *V.amygdalina* for its anti-diarrheal activity in castor oil induced diarrheal models.

Materials and Methods

Animals

Healthy adult female albino rats (150-200g) were obtained from animal house of Physiology Department, LadokeAkintola University of Technology, Ogbomoso, Oyo State. The animals were housed in cages under standard conditions of temperature

(25 + 2^{O}C), 12hr/12hr light/dark cycle and fed with standard pellet diet and water *ad libitum*. The animals were allowed to acclamatise for two weeks before the experiment. The animals were handled in accordance with the National Institute of Health (NIH) Guide for the care and use of Laboratory Animals (National Research Council, 1985) as also obtained in my institution.

Drugs and Chemicals

Castor oil, ethanol and activated charcoal were obtained from Sigma Aldrich Chemicals, Germany while Loperamide and Atropine sulphate used as standard anti-diarrheal drugs were obtained from Richy Gold international Ltd, Nigeria, a product of Laborate Pharmaceuticals, India . All reagents and chemical used were of pure analytical grade.

Plant collection and Preparation of the Extract

The stem bark of *V. amygdalina* were collected from a local farm in Aroje, Ogbomoso, Oyo State and were identified and authenticated at the botanist unit of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. The plant material was air dried at room temperature for three weeks and pulverized into powdered form with the aid of a pulverizer. 1kg of the powdered material was macerated with 5.0 litres of ethanol in a round bottom flask for two weeks with constant shaking. The resultant mixture was filtered using Whatman (No 1) filter paper and the filtrate was concentrated using a rotary evaporator and dried on a water bath to obtain a semisolid mass (yield weight 52.50g) which was stored in a desicator until used.

Preliminary Phytochemical Screening

The ethanol extract was tested for the presence or absence of alkaloids, saponins, tannins, steroids, flavonoids and phenols based on standardized methods (Trease, 1996).

Castor oil induced diarrheal

The anti-diarrheal activity of the ethanol extract was evaluated according to the method described by Teke *et al*., (2007). Female albino rats (150-200g) were divided into five groups of six animals each. They were fasted for 24hours prior to the test but allowed free access to water. Group 1 (control) was treated with 0.2ml of normal saline which served as control. Group 2 received standard drug (Loperamide, 3mg/kg). Groups 3, 4 and 5 received different doses of the extract (100, 200 and 400mg/kg) respectively. All doses were administered orally. The animals were then housed singly in cages lined with transparent paper. One hour after pretreatment with the standard drug and the extract, the animals were challenged with 1ml of castor oil orally. Thereafter, they were observed carefully every hour for 4hours for the presence of diarrhea defined as watery (wet) stools.

Study of small intestine transit

This was done according to the method described by Mujumdar, (1998). Female rats (150-200g) were divided into five groups of six animals each. They were fasted for 24 hours prior to the test but were allowed free access to water. Group 1 (control) was orally administered with 0.2ml normal saline. Group 2 orally received atropine sulphate (5mg/kg). groups 3, 4 and orally received different doses of the extract (100, 200 and 400mg/kg). Thirty minutes after drug administration, 1ml of charcoal meal (5% activated charcoal in 10% aqueous tragacanth) was administered to all the animals in the study and thirty minutes later, all the rats were sacrificed and the abdomen opened. The small intestine was dissected out and the distance covered by the charcoal from the pylorus to the caecum was measured and expressed as a percentage of the distance travelled an indices of the small intestine transit.

Castor oil induced enteropooling

This was determined as described by Havagiray *et al*., (2004). Female rats
(150-200g) were divided into five groups of six animals each and were fasted for 24 hours but allowed free access to water. Group 1 (control) was treated with 0.2ml normal saline. Group 2 was treated with the standard drug (Loperamide , 3mg/kg). Groups 3, 4 and 5 were treated with different doses of the extract (100, 200 and 400mg/kg) respectively. All administered by oral route. Thirty minutes later, all the rats were challenged with 1ml of castor oil orally. After thirty minutes, each rat was sacrificed and the small intestine was excised and the intestinal content was squeezed quantitatively into a measuring cylinder. The volume and mass of intestinal content were obtained and the inhibition of intestinal content was compiled.

Statistical analysis

Results were expressed as mean ± SEM. The significance of difference between the control and treated groups were determined using one way analysis of variance (ANOVA) followed by student's t-test and $p < 0.05$ were considered statistically significant (Kukami, 1999)

Results

Phytochemical Screening

Phytochemical analysis of the extract was positive for the presence of alkaloids, flavonoids, saponins, phenols, tannins, while steroid was not detected.

Castor oil induced diarrheal

The ethanolic extract of stem bark of *V. amygdalina* was found to be effective in a dose dependent manner against castor oil induced diarrhea in experimental animals. Pretreatment of rats with ethanolic extract of *V. amygdalina* (100, 200 and 400kg/mg) dose dependently and significantly reduced the frequency of defeacation and the total number of wet faeces produced upon administration of castor oil when compared with the control and this result is similar to the effect of the standard anti-diarrheal drug, Loperamide (3mg/kg) (Table 2). The corresponding reduction in percentages of faecal matter at 100, 200 and 400mg/kg of the extract were 64.50%, 74.20 and 88.70% respecvtively. Loperamide (3mg/kg), the standard anti-diarrheal drug inhibited the diarrhea by 89.20%.

Gastro intestinal transit

The extract of *V. amygdalina* significantly ($P < 0.05$) decreased the propulsion of charcoal meal in the rat's gastro intestinal tract at oral doses of 100, 200 and 400mg/kg respectively compared with the control group receiving normal saline. The standard anti-diarrheal drug atropine sulphate (5mg/kg) produced a marked decrease in the propulsive movement and the intestinal length travelled by charcoal. The 100, 200 and 400mg/kg of the extract produced percentage intestinal transit of 28.60%, 33.40% and 47.20% respectively while atropine sulphate produced percentage intestinal transit of 65.70% (Table 3)

Castor oil induced enteropooling

Enteropooling is the accumulation of fluid and electrolyte in the intestinal loop. Castor oil caused accumulation of fluid and electrolyte in the intestinal loop. This was apparent in all the animals after administration of castor oil but was inhibited by the ethanolic extract of *V. amygdalina*. The extract at 100, 200 and 400mg/kg showed significant ($P < 0.05$) reduction in the intestinal fluid accumulation of 26.80%, 42,90% and 60,10% respectively. The standard drug, Loperamide (5mg/kg) also significantly ($P < 0.05$) inhibited intestinal fluid accumulation by 74.40% (Table 4)

Table 1: Phytochemical constituents of the ethanolic stem bark extract of *Vernonia amygdalina*

Constituent	Observation
Alkaloids	+
Saponins	+
Tannins	+
Steroids	-
Flavonoids	+
Phenol	+

+ = present

- = absent

Table 2: Effect of ethanolic stem bark extract of *V. amygdalina* on castor oil induced diarrhea

Groups	Dose (mg/kg)	Mean weight of stools ± SE after 4 hours	% inhibition of defeacation
I.	Saline (0.2ml)	8.69 ± 0.36	
II.	Loperamide (3mg/kg)	0.94 ± 0.65**	89.20
III.	VAEE (100mg/kg)	3.06 ± 0.59**	64.50
IV.	VAEE (200mg/kg)	2.24 ± 0.74**	74.20
V.	VAEE (400mg/kg)	0.98 ± 0.63**	88.70

Values are expressed as mean ± SEM, n= 6, **P < 0.05 when compared with control group, VAEE = *Vernonia amygdalina* ethanolic extract

Table 3: Effect of ethanolic stem bark extract of *V. amygdalina* on small intestinal transit of rats

Groups	Doses (mg/kg)	Mean Distance travelled by charcoal	% inhibition
I.	Saline (0.2ml)	75.40 ± 0.12	_
II.	Atropinesulphate (5mg/kg)	25.84 ± 0.33**	65.70
III.	VAEE (100mg/kg)	53.80 ± 0.92**	28.60
IV	VAEE (200mg/kg)	50.25 ± 1.05**	33.40
V	VAEE (400mg/kg)	39.80 ± 1.24**	47.20

Values are expressed as mean ± SEM, n=6, **P < 0.05 when compared with control group, VAEE = *Vernonia amygdalina* ethanolic extract

Table 4: Effect of ethanolic stem bark extract of *V. amygdalina* on castor oil induced enteropooling

Groups	Doses (mg/kg)	Volume of intestinal content (ml)	Weight of intestinal content (g)	% inhibition in weight of intestinal content
I.	Saline (0.2ml)	3.50 ± 0.39	6.09 ± 0.27	_
II	Loperamide (3mg/kg)	1.20 ± 0.34**	1.56 ± 0.33**	74.40
III	VAEE (100mg/kg)	2.42 ± 0.53**	4.46 ± 0.45**	26.80
IV.	VAEE (200mg/kg)	1.86 ± 0.88**	3.48 ± 0.78**	42.90
V	VAEE (400mg/kg)	1.06 ± 1.40**	2.45 ± 1.09**	60.10

Values are expressed as mean ± SEM, n = 6, **P < 0.05 when compared with control group, VAEE = *Vernonia amygdalina* ethanolic extract

Discussion

Diarrheal is a very common ailment and national problem in many tropical countries and the cause of 4-5 million deaths throughout the world annually (Synder and Merson, 1982 ; Abdullahi *et al*., 2001). Diarrhea is usually considered as a result of altered motility and fluid accumulation within the intestinal tract which may be due to a disturbance in the transport of water and electrolyte in the intestine. Various medications are available for diarrhea such as loperamide, bismuth subsalicylate and racecadotril But these drugs possess many side effects such as abdominal discomfort, dry mouth, nausea, constipation and headache. Apart from modern medical therapy, the use of herbal drugs in the treatment of diarrhea diseases is a common practice in many countries including Nigeria. A number of medicinal plants have been reported to be effective against diarrhea as they are used in traditional herbal practice. Indigenous plants used for this purpose are : *Alchornea cordifolia* (Agbor *et al*., 2004), *Acacia nilotica* , *Bombax buonopenze* , *Vinca major* , *Carotheca semanoides* (Yakubu *et al*., 2012) and others. The present study was undertaken to substantiate out the scientific rationale behind the local use of stem bark of *V. amygdalina* in diarrhea. The anti-diarrheal activity of the ethanol extract of the stem bark of *V. amygdalina* was evaluated by employing castor oil induced diarrhea, gastro intestinal motility and castor oil induced enteropooling methods. Castor oil has been widely used in diarrhea studies because it is metabolized into ricinoleic acid in the gut which in turn irritates and causes inflammation in the intestinal mucosal resulting in the release of inflammatory mediators such as prostaglandins, histamine and so forth. The prostaglandins released promote vasodilation, smooth muscle mucus contraction and mucus secretion in the small intestines. Prostaglandins E are considered to be good diarrheogenic agents in experimental animals as well as in human beings. The inhibitors of prostaglandins biosynthesis are therefore considered to delay castor oil-induced diarrhea (Brijesh *et al*., 2009). The result of the present study showed that the ethanolic stem bark extract of *V. amygdalina* at 100, 200 and 400mg/kg produced a statistically reduction in the severity and frequency of diarrhea produced by castor oil when compared with untreated control and the anti-diarrheal effect of the plant extract was comparable to the standard drug loperamide which at present is one of the most efficacious and widely employed anti-diarrheal drug. Loperamide effectively antagonizes diarrheal activity induced by castor oil . In the evaluation of intestinal transit, atropine sulfate was used as standarddrug. Atropine is known to inhibit the intestinal transit propably due to its anticholinergic effect (Izzo *et al*., 1999). The etanolic stem bark extract of *V. amygdalina* reduced intestinal propulsive movement in the charcoal meal treated model at 100, 200 and 400mg/kg dose though this was not comparable with atropine sulphate. Atropine sulphate is known to produce anticholinergic effect on the intestinal transit whereas activated charcoal can prevent the absorption of drugs and other chemicals into the body by absorbing them on the surface of the charcoal particle (Venkatesan *et al*., 2005). Thus the suppression or reduction in the intestinal propulsive movement of the charcoal meal by all doses of the extract suggest among others that the extract was able to increase time for absorption of water and electrolyte

(Teke *et al*., 2007) which may indicate a reduction in the peristaltic activity and ultimate reduction in the gastro intestinal motility (Nwiniyi *et al.*, 2004) . In the enteropooling study, the ethanol stem bark extract of *V. amygdalina* significantly reduced the intestinal content of rats. The intra luminal fluid accumulation was significantly blocked by the extract . The accumulation of intestinal fluid may be a resultant clinical effect of bowel function disturbance, in which case, there is impaired intestinal absorption, excessive intestinal secretion of water and electrolytes and a rapid bowel transit (Gurgel *et al*., 2000 ; Mbagwe and Adeyemi, 2008). The reduction in the parameters of enteropooling and consequent increase in the percentage inhibition of intestinal content of the animals suggested that the extract might have inhibited or reduced the massive secretion of water into the intestinal lumen. Castor oil caused induction of diarrhea by decreasing or inhibiting the activity of Na^+ - K^+ ATPase in the small intestine and colon and thus affect electrolyte transport by reducing active Na^+ and K^+ absorption.(Palombo, 2006). Elevation of nitric oxide concentration have also been implicated in pathogenesis of diarrhea. Therefore increase in the activity of Na^+ - K^+ATPase as well as decrease in the concentration of nitric oxide in the small intestine by the extract treated animals may be one of the mechanism by which the extract exhibits its anti-diarrheal properties. Anti-diarrheal properties of medicinal plants were found to be due to the presence of tannins, flavonoids, saponins, alkaloids, sterols, reducing sugars and triterpenes (Inayathulla *et al.,* 2010). Phytochemical screening of the plant extract in this study revealed the presence of alkaloids, saponins, tannins, flavonoids, thus these chemical constituents, may be responsible for the *in vivo* anti-diarrheal activity of *V. amygdalina* stem bark extract. Flavonoids have anti-diarrheal activity which have the ability to inhibit intestinal motility and hydroelectric secretions which are known to be altered in diarrhoeic conditions (Venkatesan *et al*., 2005). Tannins present in anti-diarrhoeal plants denature the protein tannates which make the intestinal mucosa more resistant to chemical alteration and hence reduce secretion (Mohammed *et al*., 2009).

Conclusion

In conclusion , from this study , *V. amygdalina* stem bark extract possess significant anti-diarrheal activity which is mediated through inhibition of typical indices such as faecal parameters, gastro intestinal motility. Enteropooling and stimulation of Na^+ - K^+ ATPase activity and reduction in the nitric oxide concentration of the small intestine. Properties exhibited by the extract are suggestive of the possible bioactive constituents present in the extract which may possess antioxidant properties and a possible correlation with the anti-diarrheal activity shown by *V. amygdalina* , a property which could be exploited in drug design towards the treatment and management of diarrhea.

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NO CONFLICTS OF INTEREST EXIST AMONG AUTHORS OF THIS MANUSCRIPT.

REFERENCES

1.Mathan ,V.I. (1998) : Diarrhoeal diseases. Department of Gastro Intestinal Sciences. Christian Medical College and Hospital, Vellore, India. *British Medical Bulletin* ; 54 (2) : 407- 419.

2. Guerrant R.L., Van Gilder T., Steiner T.S., Theiman M.N., Slutsker L., Tauxe R.V. (2001) : Practise , guidelines for the management of infectious diarrhea *Clinical Infectious Disease* ; 32 : 331- 335.

3. Toyin Y.M., Khadjat C.F., Saoban S.S., Olakunle A.T., Abraham B.F., Luqman O.A (2012) : Anti-diarrheal activity of aqueous leaf extract of *Ceratotheca sesamoides* in rats. *Bangladesh Journal of Pharmacology* ; 7 : 14 - 20.

4. Robert K., Egon S., Daniela B., Florian D., Christoph W., Gunter J.K., Emil C.R. (2001) : Role of Candida in antibiotic associated diarrhea. *Journal of Infectious Disease* ; 184 : 1065 – 1069.

5. Shoba F.G and Thomas M. (2001) : Study of anti-diarrheal activity of four medicinal plants in castor oil induced diarrhea. *Journal of Ethnopharmacology* ; 76 : 73 – 76.

6. Atta A.H and Mouneir S. M. (2004) : Anti-diarrheal activity of some Egyptian medicinal plant extracts. *Journal of Ethnopharmacology* ; 26 : 101 – 109.

7. Aregheore E.M.K.,Makkar H.P.S., Becker K. (1998) : Feed values of some browse plants from the central zone of Delta State . *Niger .Trop.Science* ; 38 (2) : 97- 104.

8. Allen G.H (1987) : The genetic basis of disease in general pathology

9. Burkhill H.M (1985) : The useful plants of West Tropica. Africa Vol 2, Families A.D Kew Royal Botanic Gardens.

10. Chall S and Willcox M. (2009) : A clinical trial of the traditional medicine *Vernonia amygdalina* in the treatment of uncomplicated malaria. *Journal of Alternative and Comlpementary Medicine* ; 15 (11) : 1231 – 1237.

11. Akah P.A and Okafor C. (1992) : Hyperglycemic effect of *Vernonia amygdalina* . Del on experimental

rabbits. *Plant Medical Research* ; 1 : 6 – 10.
12. Swee K.Y., Wan Y.H.T.,Boon K.B.,Woonl S.L., Huynth K.Y., Abdul H., Noaman Yand Noorjaham B. A.(2010) : *Vernonia amygdalina* an ethnovetenary and ethnomedical used green vegetable with multiple bioactivities. *Journal of Medicinal Plant Research* ; 4 (25) : 2787 – 2812.
13. National Reseach Council Washington DC (1985). The National Academic Press. STJ.
14. Trease G.E., Evans MC editors (1996) : Textbook of Pharmacognosy 14th ed. London. Bailliere Tindal pg 565 – 566.
15. Teke N. G., Kulate J.R., Ngouaten O.B., Getsung D. (2007) : Anti-diarrheal and antimicrobial activities of *Emilia coccinea* (Sims) G extracts. *Journal of Ethnopharmacology* 112 : 278 – 283.
16. Mujumdar A.M. (1998) : Antia-diarrheal of *Azadirachta indica* leaf extract . *Indian Drugs* ; 35 : 417 – 420.
17. Havagiray R., Ramesh C., Sadhna K. (2004) : Study of anti-diarrheal activity of *Calotropis g igantea* R.B.R in experimental animals. *Journal of Pharm. Pharmaceut. Sci*. ; 7 : 70 – 75.
18. KuKami S.K. (1999) : Handbook of experimental pharmacology, Vallabh Prakashan, New Delhi, India, 172
19. Synder J.D and Merson M.H. (1982) : The magnitude of the global problem of acute diarrhea disease. A review of active surveillance of data. Bulletin WHO ; 60 : 605 – 613.
20. Abdullahi A.L., Agbo M.O., Amos S., Gamaniel K.Sand Wambebe C. (2001) : Anti-diarrheal activity of the aqueous extract of *Terminalia avicennoides* roots. *Phythotherapy Research* ; 15 : 431 – 434.
21. Agbor G.A., Leopoid T., Jeanne N.Y. (2004) : The anti-diarrheal activity of *Alchornea cordifolia* leaf extract. *Phythotherapy Research* ; 18(11) : 873 - 876.
22. Yakubu M., Toyin O.F., Khadijat S.S., Ajiboye T.O., Bamisaye F.A. and Quadri A.L. (2012) : Anti-diarrheal activity of aqueous leaf extract of *Ceratotheca sesamoides* in rats. *Bangladesh Journal of P harmacology* ; 7 : 14 – 20.
23. Brijesh S., Daswani P., Terali P., Antia N., Birdi T. (2009) : Studies on the anti-diarrheal activity of Aegie marmedos unripe fruit. Validating its traditional usage.*BMC Complement Altern. Med* ; 9(47) : 1- 12.
24. Izzo A.A., Mascolo R.C., Germano M.P., De pasquale R and Capasso F (1999) : Inhibitory effect of cannabinoid agonist on gastric emptying in rats. *Archives of Pharmacology* ; 360 : 221 – 223.
25. Venkastesan N., Vadivu T., Sathiya N., Arokya A., Sundaranjan R., Sengodan G., Vijaya K., Thandavarayan R., James B.P .(2005) : Anti-diarrheal potential of*Asparagus racemosus* wild root extracts in laboratory animals. *Journal of Pharmaceutical Science* ; 8 : 39 – 45.
26. Nwinyi F.C., Binda I., Ajoku G.A., Aniagu S.O., Enwerem N.M., Irisadipe A.,Kubmarawa D., Gamaniel K.S. (2004) : Evaluation of the aqueous extract of *Boswellia dalzielii* stem bark for antimicrobial activities and gastrointestinal effects.*African Journal of Biotechnology* ; 3 : 284 – 288
27. Gurgel L.A., Silva R.M., Santos F.A., Martins D.T.O., Mattos P.O., Rao V.S.N. (2001) : Studies on the anti-diarrheal effect of dragon's blood from *Croton urucarana*. *Phytother. Res* ; 15 : 319 – 322.
28. Mbagwu H.O.C., Adeyemi O,O (2008) : anti-diarrheal activity of *Mezoneuron benthamianum* Baill (Caesalpinaceae). *Journal of Ethnopharmacology* ; 116 : 16 – 20.
29. Palombo E.A. (2006) : Phytochemicals from traditional medicinal plants used intreatment of diarrhea : Modes of action and effects on intestinal function. *Phythother Res* ; 20 : 712 – 724.
30. Inayathulla, Sherrif W.R., Karigar A.A., Sikarwar M.S. (2010) : Evaluation of anti-diarrheal activity of *Crataeva nurvala* root bark in experimental animals. *Int. J.Pharm Pharm Sci* ; 2(1) : 158 – 161.
31. Mohammed A., Ahmed H., Goji A.D.T., Kpanachi A.O., Ezekiel I., Tanko Y (2009) : Preliminary activity of hydro methanolic extract of aerial part of *Indigofera pulchra* in rodents. *Asian J.Med.Sci* ; 1(2) : 22 – 25.