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Phytochemical Screening and Evaluation of Anti-diarrhoeal Property of *Astilbe rivularis* in Albino Wistar Rats

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Abstract: Introduction: Diarrhea is characterized by increased water content of the feces, which is usually accompanied by increased frequency of defecation. Diarrhea may be defined as a condition in which fecal mass and its water content are greater than usual. *Astilbe rivularis* perennial herb about ultimate height around 0.6-2.5 m tall. It is found in Nepal and Vietnam. It is widely distributed in the high mountains areas at 2000-3600 m. All parts of plant are aromatic in odor. In the *Astilbe rivularis* plants, flavonoids, tannins, saponins, polyphenols, terpenoids are present whereas coumarins is absent. **Objective:** To perform phytochemical screening and evaluation of anti-diarrhoeal property of *Astilbe rivularis* in albino wistar rats. **Materials and Methods:** Dried rhizomes of *Astilbe rivularis* was extracted with ethanol (99.96%) by using Soxhlet apparatus. Castor oil-induced diarrhea was used to evaluate the anti-diarrheal activities of extract in wistar rat. Each experiment consisted of 30 animals randomly but equally divided into groups. Group A was taken as control & the Group B was taken as standard drug Loperamide (2 mg/kg). The ethanol extract of herbal formulation of *Astilbe rivularis* rhizomes 100 mg/kg were feed to Group C, 200 mg/kg were feed to Group D and 400 mg/kg were feed to Group E respectively. The data were analyzed by using One-way ANOVA. The ethanolic extract of *Astilbe rivularis* was used to evaluate the gastrointestinal motility by using the enterpooling in the wistar rats. Likewise, the acute toxicity of the drug was evaluated by using the ethanolic extract of *Astilbe rivularis* in the wistar rat. For the acute toxicity, 2000 mg/kg ethanolic extracts of *Astilbe rivularis* were given to ten wistar rat for experimental study. **Observation and Results:** The herbal formulation has significantly decreases the frequency of diarrhea induced by castor oil in wistar rats as compared to the control & standard drug group. Castor oil induced enteropooling in rats when tested at 100 mg/kg, 200 mg/kg and 400 mg/kg respectively. The phytochemical screening of the ethanolic extract of *Astilbe rivularis* revealed the presence of flavonoids, tannins, saponins, triterpenes, phenols and alkaloids.

Key words: *Astilbe rivularis*, anti-diarrhoeal, castor oil, loperamide, ethanol, wistar.

1. Introduction

A sizable section of the populace in developing nations like Nepal uses traditional medicine and medicinal herbs to address their health care needs. Whereas herbal medicines and traditional medicines are popular for historical and cultural reasons [1].

By exploring the human aspects of medicine and mapping the development of medical theories, policies and institutions across time, medical history can reveal the reflects and shapes far wider for historical currents which extent the experiences of health and disease structure of our lives [2].

Diarrhea is defined as having at least three loose, liquid or watery bowel movements that often last for a few days and cause dehydration due to fluid loss or electrolytes in the body. There is an increase in secretion and a decrease in fluid absorption, as well as electrolyte and water loss. It is also characterized by the discharge of semi-solid or watery fecal matter from the bowel three or more times per day. Globally in the present state nearly 1.7 billion cases of childhood diarrheal disease is caused every year. Each

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year 525,000 children under five years are killed [3].

Many synthetic drugs like Diphenoxylate, Loperamide, Clonidine, Kaolin, Pectin, Bismuth Subsalicylate, Cholestyramine, Cholestipol and antibiotics are also available for the treatment of diarrhea [4]. Antibiotics used for the treatment of diarrhea are Ampicillin, Amoxicillin–Clavulanate, Cefixime, Cephalosporins, Fluoroquinolones, Azithromycin, Clarithromycin, Erythromycin and Tetracycline. Loperamide appears to be well tolerated but has side effects of gastro intestinal in nature such as nausea, vomiting, abdominal cramping, dizziness, rash and dry mouth [5]. Diphenoxylate have some unpleasant side effects like tachycardia, dry mouth, nausea, changes in mood, numbness in arms & legs, loss of appetite and blurring of vision etc [6]. Norfloxacin is used for the treatment of diarrhea and have side effects such as seizures, photosensitivity, rashes, depression, anxiety, hypoglycemia, hypoglycemia, dizziness muscle spasm, headache and confusion [7].

Astilbe rivularis is a perennial herb (belonging to family Saxifragaceace) around 0.6-2.5 m tall and leaves are pinnately compound. The leaflets measuring (4-14.5) cm and (1.7-8.4) cm are elliptic to ovate and acuminate. Base is cordate or rounded to cuneate, margin doubly serrate. They have axially brown long pilose and glandular hairy along veins. The panicle is around 42 cm with many flowers and braches measures 1-18 cm and are brown, crisped, glandular and hairy [8].

The flowering season is July to October and the flowers are yellowish white with 5-10 stamens and 2 carpels. Base is cannate having sub superior ovary. Sepals are 4 or 5, green, ovate or elliptic to oblong. Petioles have long brown hairs, especially at the point of attachment of the leaflets. Stems are long brown glandular and hairy. Fruiting season is October to November. Fruits is an ovoid capsule and bears ellipsoidal seeds at their end whereas germination is by vegetative method, it also germinates via seeds in presence of moist and light within 1 to 4 weeks at 15 ℃. Roots are thick, hairy and rhizomatous [9].

Astilbe rivularis contains alkaloids, tannins, flavonoids, coumarins, and glycosides. The importance of *Astilbe rivularis* is reflected by the fact that all of its parts have ethno medicinal value. Root is valued during diarrhea, dysentery, prolapse of the uterus and hemorrhage. Rhizome paste along with honey is taken to control postpartum bleeding, diarrhea and dysentery. Also, the root bark is used in body ache and menstrual disorder. Leaves are used as blood purifier [10].

Botanical Name: *Astilbe rivularis*

Synonyms: River astilbe

Common Names: Thulo okhati, Budo okhati

It is a Perennial Herb and can be identified with height 1 to 1.5 m tall, pinnately compound leaf, with an ovoid capsule fruits and ellipsoidal seeds [11].

2. Material and Method

2.1 Plant Materials

2.1.1 Collection and identification of plant materials

The rhizomes of *Astilbe rivularis* were obtained from Daman, Makwanpur in September, 2019. The plant was taxonomically identified by Ms.Til Kumari Thapa, Department of National Herbarium and Plant laboratories, Godawari, Lalitpur.

2.1.2 Drying

About 2 kg fresh *Astilbe rivularis* rhizomes were manually separated from whole plants, washed thoroughly with tap water and rinsed in distilled water. Rhizomes were chopped in small sizes and air-dried in the shade at room temperature for about a month. Weight of rhizome after drying were 350-400 gms of *Astilbe rivularis*.

2.1.3 Extraction of plant materials

183 gms of the powdered sample were extracted using ethanol (99.99%) in Soxhlet apparatus for 18-24 hours at 78 ℃ through a regulated heating mantle. The obtained extracts were concentrated under 70 ℃

using a rotary vacuum evaporator till solvent fully evaporated. The obtained extracts were stored in refrigerator for further usage.

2.2 Screening of Phytochemicals.

2.2.1 Qualitative analysis of phytochemical constituents

The phytochemical screening of *Astilbe rivularis* rhizome extract were carried out to confirm the presence of secondary metabolites for alkaloids, tannins, phenolic compounds, glycosides, triterpenes, saponins, steroids, coumarins and flavonoids by using standard protocols [12]

2.2.2 Detection of flavonoids

1) Ferric chloride test:

Extract was treated with few drops of neutral ferric chloride solution. Formation of green color detect the presence of flavonoids.

2) Lead acetate test:

Extract was mixed with few drops of 10% lead acetate. Formation of yellow precipitate indicated the presence of flavonoids.

3) Shinoda test:

Extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet color appeared after few minutes which indicated the presence of flavonoids.

2.2.3 Detection of alkaloids

The extract were mixed with ammonia and then extracted with chloroform solution. To this, dilute HCl was added. The acid layer were used for chemical tests for alkaloids.

1) Mayer's test (Potassium Mercuric Iodide):

The acid layer with few drops of Mayer's reagent gives a creamy white precipitate which indicates the presence of alkaloids.

2) Hager's test (Saturated solution of picric acid):

The acid layer with Hager's reagent gives yellow precipitation which detect the presence of alkaloids.

3) Dragendroff's test (Solution of Potassium Bismuth Iodide):

The acid layer with few drops of Dragendroff's reagent give reddish brown precipitate which indicate the presence of alkaloids.

2.2.4 Detection of tannins

- Gelatin Test:

1% of gelatin solution containing sodium chloride was added to the extract. Formation of white precipitate indicated the presence of tannins.

2.2.5 Detection of glycosides

Extracts was hydrolysed with dilute HCl, and then subjected to test for glycosides.

- Legal's test:

To 2 ml of extract with dilute HCl and 2 drops of Sodium nitroprusside in few ml of pyridine and 20% sodium hydroxide was added. Formation of pink to blood red color indicated the presence of Cardiac glycosides.

2.2.6 Detection of phenols

For chemical test of phenol, the extract were dissolved in water.

1) Ferric Chloride test:

Extract was treated with 3-4 drops of 5% ferric chloride solution. Formation of deep blue black color indicated the presence of phenols.

2) Lead Acetate test:

Extract was treated with 3ml of 10% lead acetate solution. A bulky white precipitate detected the presence of phenolic compounds.

2.27 Detection of saponins

- Foam test:

0.5 gm of extract was shaken with 2ml of water. If foam produced stable for ten minutes, it indicated the presence of saponins.

2.2.8 Detection of triterpenes

1) Tschugajen test:

Chloroform solution of the extract with excess of acetyl chloride and pinch of zinc chloride and warming on water bath gives Eosin red color.

2) Salkowski test:

Chloroform solution of the extract when shaken with concentrated sulphuric acid, lower layer turns to

yellow on standing which detect the presence of triterpenes.

2.2.9 Detection of steroids

1) Salkowski test:

Chloroform solution of the extract when shaken with concentrated sulphuric acid and on standing gives red color indicated the presence of steroids.

2) Liberman Buchardt test:

Chloroform solution of the extract with few drops of acetic acid and 1 ml of concentrated sulphuric acid from the sides gives reddish ring at the junction of 2 layers which detected the presence of steroids.

2.2.10 Detection of coumarins

Extract solution was concentrated to yield a residue. The residue is dissolved in hot water. After cooling solution was divided in two test tubes. To one test tube 10% (w/v) Ammonium Hydroxide was added and to the other test tube was used as control. Fluorescence color indicates the presence of coumarins.

2.2.11 Detection of Reducing sugar (Fehling's test)

Fehling A and Fehling B reagents was taken in equal volume were mixed together and 2 ml of it was added to crude extract and gently boiled. A brick red precipitate was appeared at the bottom of the test tube which indicated the presence of reducing sugars.

2.2.12 Experimental animal

Wistar rats of both sexes were used for the studies. These rats were obtained from the Om Sairam Enterprises, Tripureshwor, Kathmandu, Nepal. The animals were housed in cages under standard laboratory conditions (12:12 hour light/dark cycle at 25 ± 2 ℃). They had free access to the standard commercial diet and water. The animals were divided into groups of five and fasted for 18 hours before the experiment. The ethical guidelines for the investigation of animals used in experiments were followed in all tests.

2.2.13 Experimental design

Experimental animals were randomly selected and divided into five groups denoted as Group-A, Group-B, Group-C, Group-D and Group-E consisting of 5 rats in each group. Each group received a particular treatment i.e. Control, Standard drug and a dose of the *A. rivularis* extract at 100 mg/kg, 200 mg/kg and 400 mg/kg. Each animals was weighed properly and the dose of the test samples and control materials were adjusted accordingly.

3. Acute Toxicity Study

For acute toxicity study, *A. rivularis* rhizome extract was administered orally for about 2000 mg/kg to the albino Wistar rats employing the fixed dose method as per Organization for Economic Co-operation and Development (OECD) guideline. And the intake of water and food, mortality, signs and symptoms of toxicity for 48 h and then up to 12 days was recorded [13]. Before the administration of test substance, individual weight of the animals should be recorded and thereafter weekly. The change in the weight should be calculated and recorded. At the end, surviving animals are humanly killed. The number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test or killed for humane reasons, time of death of individual animals, a description and the time course of toxic effects and reversibility should be calculated and recorded too [14].

3.1 Preparation of Herbal Formulation Doses

Herbal formulation doses were prepared in 10% CMC (CarboxyMethyl Cellulose) as a solution and administered to the respective doses.

3.2 Methods for Evaluating Anti-diarrheal Agents (In-Vivo)

3.2.1 Castor oil induced diarrhea

Wistar albino rats weighing 180-300 gm were used. Animals were divided inti five groups of four animals each as Group I, Group II, Group III, Group IV and Group V. Animals were fasted for 18 hrs. and allowed freely access to water before experiment [15]. And

received castor oil at a dose of 1 ml/animal orally (p.o.) using feeding tube for induction of diarrhea. Thirty minutes after castor oil administration, rats of group I (control) received 1.0 ml/100 g of 0.9% NaCl in distilled water (normal saline) and rats of groups II received standard drug, loperamide (2 mg/kg p.o.) Group III, IV and V received 100, 200 and 400 mg/kg EEAR p.o. respectively. The animals were placed separately in metabolic cages over white clean Whatman filter paper, which was changed every hour. The severity of diarrhea was assessed each hour for 4 hours. The total number of diarrhea feces of the control group was considered 100%.

% inhibition = (Control-Test) × 100/Control [16]

3.3 Castor Oil-induced Enteropooling

Animals were divided into five groups of four rats each. They were fasted 18 hrs prior to the experiment. Group I (controls) was treated with 1 ml of normal saline. Group II was treated with standard drug (Loperamide 2 mg/kg). Group III, IV and V was treated with different doses of the extract (100, 200 and 400 mg/kg) respectively, one hour before the oral administration of castor oil (1 ml/rat). All were administered by the oral route. After 4 hrs. Each rats were sacrificed [17]. And the whole length of the intestine from pylorus to caecum was dissected out. Then content in the intestine were collected in measuring cylinder and the volume were measured out [18].

4. Results

- Phytochemical Screening

The phytochemical screening of ethanolic extract of rhizome *Astilbe rivularis* were done, the result of which is shown in Table 1.

Table 1 Quantitative analysis of phytochemical constituents.

S.N	Phyto Constituents	Test/Reagents	Ethanolic extract of rhizome
1.	Flavonoids	Ferric Chloride Lead Acetate	+ +
2.	Alkaloids	Mayer's Hager's Dragendroff's	+ + +
3.	Tannins	Gelatin	+
4.	Phenol	Feric Chloride Lead Acetate	+ +
5.	Saponins	Foam	+
6.	Triterpenes	Salkowski Tschugajen	+ +
7.	Steroids	Salkowski Liberman Burchardt	+ +
8.	Coumarin		_
9.	Glycosides	Legal's	+
10.	Reducing Sugar	Fehlings A & B	+

(-) sign denotes the absence of a constituent in the respective screening test; (+) sign denotes the presence of a constituent in the respective screening test.

- Acute toxicity studies (LD_{50})

Ethanolic extract of *Astilbe rivularis* rhizome was studied for acute toxicity at dose of 2000 mg/kg by oral route.

The extract was found to be 90.02 gm. In our experimental study, following physical appearance are observed as swelling of limbs, swelling of stomach, loss of weight and less consumption of meal and less movement. They were observed for 12 days and they were sacrificed.

By dissecting, following changes were observed as: Liver abscess, inflammation of caecum, damage of kidney, and other internal organs i.e. Spleen, pancreas, heart, lungs, were normal.

- Anti-diarrheal Activity

The antidiarrheal activity of ethanolic extract of rhizome of *Astilbe rivularis* was determined using castor-oil induced diarrhea, castor-oil induced enteropooling and acute toxicity in wistar albino rats whose result is shown in Table 2 and 3 respectively.

Table 2 Effect of ethanolic extract of *Astilbe rivularis* rhizome (EFAR) at different dose levels on castor oil-induced diarrhea in rats.

Group	Treatment (mg/kg)	Mean no.of diarrheal feces (gm)	% inhibition	Mean weight of feces (gm)	% inhibition
Control	-	4.75 ± 0.63	0	3.96 ± 5.95	0
Loperamide	2	1.08	78.94	1 ± 1.64	72.70
EEAR	100	4.25 ± 0.56	10.53	2.46 ± 3.7	37.87
EEAR	200	3.75 ± 0.54	21.05	2.03 ± 3.05	39.65
EEAR	400	2.25 ± 0.22	52.63	2.01 ± 22.43	49.24

All values are expressed in mean ± SEM, n = 4; there is significant difference between control and test $p < 0.001$, and $p < 0.001$.

Table 3 Effect of ethanolic extract of *Astilbe rivularis* (EFAR) at different dose levels on Castor oil induced enterpooling in rats.

Group	Treatment (mg/kg)	Volume of Intestinal content (gm)	Weight of intestinal content (gm)	% inhibition
Control	1	1.80 ± 0.65	4.26 ± 1.80	0
Loperamide	2	1.04 ± 0.20	4.55 ± 0.83	42.22
EEAR	100	1.72 ± 0.12	5.57 ± 0.91	4.97
EEAR	200	1.61 ± 0.29	6.57 ± 0.28	10.96
EEAR	400	1.37 ± 0.16	4.28 ± 0.76	23.88

All values are expressed in mean ± SEM, n = 4; there is significant difference between control and test $p < 0.001$, and $p < 0.001$.

5. Discussion

Castor oil cause changes in the intestinal mucosal membranes for water and electrolytes, resulting in decrease of water, electrolyte and fluid content in the small and large intestines [19].

Many plants consisting tannins as their constituent that indicates the anti-diarrheal activity. Tannins are responsible for the denaturation of proteins and form a complex (protein tannate) in which the intestinal mucosa becomes more resistant and reduces the secretion by virtue of which is said to be effective remedy for diarrhea [20].

The effect of the plant *Astilbe rivularis* extracts were found to be equivalent to Loperamide i.e. the drug which is widely used against diarrheal disorder which is widely antagonizes diarrhea induced by castor oil. Because of antimotility and antisecretory properties, Loperamide shows its pharmacological effects [21].

The rhizome of *A. rivularis* is used to treat a number of diseases such as stomachache, diarrhea, dysentery, headache, cough, rheumatism, peptic ulcer and malaria. Therefore, the methanolic extract of the rhizome was studied for phytoconstituents, antimicrobial and antioxidants activities. Primarily, plant water extracts are being used for their medicinal use, however, plants extracted in organic solvents have been found to give more consistent *in vitro* biological activities [22].

Ricinoleic acid, an active component of castor oil induces changes in mucosal permeability, electrolyte transport and intestinal peristalsis, which leads to hyper secretory of the intestinal mucosa, leading to prostaglandin's release that cause an increase in net secretion of water and electrolytes into the small intestine. Ricinoleic acid causes irritation and inflammation biosynthesis delay castor oil induced diarrhea. Tannins shows an antidiarrheal effect and these substances may precipitate proteins of the

electrolytes, reduce peristaltic movement and intestinal secretion. The anti-diarrheal activity of flavonoids has been described to their ability to inhibit intestinal motility and hydro electrolytic secretion that is known to be altered in intestinal condition [23].

6. Conclusions

The study was focused on anti-diarrheal activities and phytochemical screening of *Astilbe rivularis* found in Gaushala, Kathmandu. The presence of flavonoids, tannins, alkaloids, and saponin. The presence of these phytochemical showed that it is source for medicinal values.

The anti-diarrheal activity was shown by the ethanolic extract in rat model which provides evidence of its use as treatment foe diarrhea in traditional medicine.

In conclusion, the results of this investigation revealed that petroleum ether extract contains pharmacologically active substance(s) with antidiarrheal properties. This provides the rationale for the use of the rhizome of *Astilbe rivularis* as an anti-diarrheal drug. Further research is to be carried out to fractionate and purify the extract, in order to find out the molecule responsible for the anti-diarrheal activity observed.

Recommendation

Phytoconstituents which are present in plant may be helpful for further investigation in anti-diarrheal further activities, acute toxicity & gastrointestinal motility, its mechanism of action and elucidation of various phytomolecules in near future.

Further study are required to get more accurate anti-diarrheal activities, acute toxicity & gastro intestinal mobility of herbal formulation of *Astilbe rivularis* as this study is conducted in small laboratory with limited equipment and rats.

The formulation is given per oral in this study so, further study is required to evaluate the anti-diarrheal activities, acute toxicity and the gastro-intestinal mobility (i.e., enteropooling) activities of herbal formulation of *Astilbe rivularis* given through other route.

Further, research is required to identify the biomolecules responsible for the anti-diarrheal activities, acute toxicity and gastro-intestinal mobility.

The study may be further explored as an anti-diarrheal, acute toxicity & gastro-intestinal remedy.

Further, preclinical study can be done in this topic in large scale so that the finding can be generalized.

It is recommended to research every constituent separately for anti-diarrheal, acute toxicity & gastro-intestinal mobility of herbal formulation.

Further studies on pharmacokinetic & pharmacodynamics properties can be done.

References

[1] Available on: https://monographs.iarc.who.int/wp-content/uploads/2018/06/mono82-6A.pdf.

[2] Jackson, M. 2014. *The History of Medicine: A Beginner's Guide*. Oneworld Publications, pp. 1-256.

[3] Snyder, J. D., and Merson, M. H. 1982. "The Magnitude of the Global Problem of Acute Diarrhoeal Disease: A Review of Active Surveillance Data." *Bull World Health Organ* 60 (4): 605-13.

[4] Abdullahi, A. L., Agho, M. O., Amos, S., et al. 2001. "Antidiarrhoeal Activity of the Aqueous Extract of *Terminalia avicennoides* Roots." *Phytother Res* 15 (5): 431-4.

[5] Heel, R. C., Brogden, R. N., Speight, T. M., & Avery, G. S. 1978. "Loperamide: A Review of Its Pharmacological Properties and Therapeutic Efficacy in Diarrhoea." *Drugs* 15 (1): 33-52.

[6] Clayton, B. D. *Mosby's Handbook of Pharmacology in Nursing* (the Fourth edition). The C. V. Mosby Company, St. Louis MO, 1987, p. 711.

[7] Sousa, J., Alves, G., Fortuna, A., & Falcão, A. 2014. "Third and Fourth Generation Fluoroquinolone Antibacterials: A Systematic Review of Safety and Toxicity Profiles." *Curr Drug Saf* 9 (2): 89-105.

[8] Han, J-H., Zhou, W., Li, W., et al. 2015. "Pentacyclic Triterpenoids from *Astilbe rivularis* that Enhance Glucose Uptake via the Activation of Akt and Erk1/2 in C2C12 Myotubes." *J Nat Prod* 78 (5): 1005-14.

[9] Mitra, P., Ghosh, T., and Mitra, P. K. 2013. "Anti-peptic

Ulcer Activity of TLC Separated Fractions of Root Extract of *Astilbe rivularis* in Rats." *European Journal of Biotechnology and Bioscience* pp. 47-52.

[10] Timalsena, S., and Lamichhane, P. P. 2019. "*Astilbe Rivularis*: Bioactive Compounds and Pharmacological Functions." *Chin J Integr Med* 25 (10): 795-9.

[11] Adhikary, P., Roshan, K. C., Kayastha, D., et al. 2011. "Phytochemical Screening and Anti-Microbial Properties of Medicinal Plants of Dhunkharka Community, Kavrepalanchowk, Nepal." *International Journal of Pharmaceutical & Biological Archives* 2 (6): 1663-7.

[12] Dhawan, D., and Gupta, J. 2017. "Comparison of Different Solvents for Phytochemical Extraction Potential from *Datura metel* Plant Leaves." *International Journal of Biological Chemistry* 11 (1): 17-22.

[13] Gupta, R., and Gupta, J. 2016. "Investigation of Antidiarrhoeal Activity of Ethanolic Extract of *Tamarindus indica* L. Seeds in Albino Wistar Rats." *Asian Journal of Pharmaceutics* 10 (4): S492-S496.

[14] Test No. 423: Acute Oral toxicity - Acute Toxic Class Method in *OECD Guidelines for the Testing of Chemicals, Section 4*, pp. 1-14.

[15] Shiramane, R. S., Biradar, K. V., Chivde, B. V., et al. 2011. "In-vivo Antidiarrhoeal Activity of Ethanolic Extract of *Delonix regia* Flowers in Experimental Induced Diarrhoea in Wistar Albino rats." *Int J Res Pharm Chem* 1 (3): 442-7.

[16] Verma, N., Singh, A. P., Gupta, A., et al. 2011. "Antidiarrheal Potential of Standardized Extract of *Rhododendron arboreum* Smith Flowers in Experimental Animals." *Indian J Pharmacol* 43 (6): 689-93.

[17] Akuodor, G. C., Muazzam, I., Usman-Idris, M., et al. 2011. "Evaluation of the Antidiarrheal Activity of Methanol Leaf Extract of *Bombax Buonopozense* in Rats." *Ibnosina J Med BS* 3 (1): 15-20.

[18] Sunder, S., Rai, A. K., Praveen, S., & Singh, P. A. 2011. "Antidiarrhoeal Activity of *Aerva lanata* in Experimentally Induced Diarrhoea in Rats." *Pharmacologyonline* 2: 921-8.

[19] Mbagwu, H. O. C., and Adeyemi, O. O. 2008. "Anti-diarrhoeal Activity of the Aqueous Extract of *Mezoneuron benthamianum* Baill (Caesalpiniaceae)," *J Ethnopharmacol* 116 (1): 16-20.

[20] Ojewole, J. A. O., Awe, E. O., and Chiwororo, W. D. H. 2008. "Antidiarrhoeal Activity of *Psidium guajava* Linn. (Myrtaceae) Leaf Aqueous Extract in Rodents." *J Smooth Muscle Res* 44 (6): 195-207.

[21] Agunu, A., Yusuf, S., Andrew, G. O., et al. 2005. "Evaluation of Five Medicinal Plants Used in Diarrhoea Treatment in Nigeria." *J Ethnopharmacol* 101 (1-3): 27-30.

[22] Adhikary, P., Roshan, K. C., Kayastha, D., et al. 2012. "In Vitro Evaluation of Antimicrobial and Cytotoxic Potential of Dry Rhizome Extract of *Astilbe Rivulari*." *International Journal of Pharmacognosy and Phytochemical Research* 4 (3): 122-6.

[23] Teke, G. N., Kuiate, J. R., Kueté, V., et al. 2010. "Antidiarrheal Activity of Extracts and Compound from *Trilepisium madagascariense* Stem Bark." *Indian Journal of Pharmacology* 42 (3): 157-163.