IJMPR 2020, 4(4), 06-11

International Journal of Modern Pharmaceutical Research

www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article

SJIF Impact Factor: 5.273

AQUEOUS EXTRACTS OF *OCIMUMGRATISSIMUM* AND *ANACARDIUM OCCIDENTALE* SYNERGISES INANTI-DIARRHOEAL PROPERTY

[1]Ihekwereme C. P., [2]*Bruce S. O., [1]Orji C. E., [2]Ibe C. I. and [3]Iloh E. S.

[1]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria.
[2]Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria,
[3]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University. Anambra State, Nigeria.

Received on: 01/06/2020
Revised on: 22/06/2020
Accepted on: 12//07/2020

***Corresponding Author**
Bruce S. O.
Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria.

ABSTRACT

Introduction: The present study was performed to substantiate the traditional claim of the anti-diarrhoeal activity of aqueous leaf extracts of *Ocimum gratissimum* and *Anacardium occidentale*, in albino mice. **Method:** Acute toxicity study was determined. The dose levels 100, 200 and 400mg/kg of aqueous extract was tested in albino miceagainst castor-oil induced diarrhoea model and charcoal meal test/intestinal motility test. The symptoms observed were the consistency of faeces, onset of defecation and cumulative faecal weight in the castor oil-induced diarrhoea model, and the distance travelled by charcoal in the intestinal motility test. **Results:** The toxicological studies confirm that the aqueous leaf extracts of *Ocimum gratissimum* and *Anacardium occidentale*, in combined form, when administered, showed no mortality or any sign of toxicity at the dose level up to 5000mg/kg. A significant delay in the onset of defecation ($p<0.05$), reduction in the cumulative faecal weight ($p<0.001$), along with a change in the faecal consistency from watery to solid form was observed at the dose of 800mg/kg in the castor oil-induced diarrhoea model. **Conclusion:** The aqueous extract of leaves of *Occimum gratissimum* and *Anacardium occidentale* showed anti-diarrhoeal activity, which may be due to its anti-motility and anti-secretory effects, which thus proved the traditional claims.

KEYWORDS: Diarrhoea; *Ocimum gratissimum*; *Anacardium occidentale*; Castor oil; Diarrhea; loperamide.

INTRODUCTION

Polyherbal medicinal formula is a common therapeutic regimen in traditional and folkloric medicine andseveral benefits have been presented in favour of it. The aetiology of diarrhoea is multifactorial and polyherbalism has the possibility of addressing all facets of it. Polyherbalism confers some benefits that are not available in a single herbal formulation, although, a better therapeutic effect can be reached with a single multi-constituent formulation. For this, a lower dose of the herbal preparation maybe required to achieve a desirable pharmacological action, thus reducing the risk of deleterious harmful side effects. Besides, Polyherbal Formulations help to improve convenience for patients by eliminating the need for taking more than one formulation at a time, bringing about better compliance (Subramani *et al.*, 2014). Other benefits of polyherbal formulations include wide therapeutic range, provision of thorough relief, effective and favourable treatment outcome (Chorgade, 2007) amongst others. The importance of using traditional or indigenous medicine for the treatment of the symptoms of diarrhoea has been encouraged by WHO in its diarrhoea control programme (Ezeigbo*et al.*, 2012). Therefore, the identification and evaluation of available natural herbs as potential alternatives to existing anti-diarrhoea drugs. The beneficial effects of medicinal plants such as *Ocimum gratissimum* and *Anacardium occidentale*, used in the traditional treatment of diarrhoeal diseases have been highlighted by various studies. *Ocimum gratissimum* (Clove basil, African basil, and Wild basil) has been reported to have medicinal attributes. Extracts of *O. gratissimum* are popular for the treatment of diarrhoea in Nigeria. Cold infusions of the leaves are usedto relieve stomach upset and haemorrhoids. The leaf is reported to be rich in thymol, which has antimicrobial properties (Iloriet *al.*, 2013). The leaves of *O. gratissimum* in traditional medicine is used for the treatment of conjunctivitis by instillation into the eyes, and as a general tonic and anti-diarrhea agent; the leaf oil when mixed with alcohol is applied as a lotion for skin infections and taken internally for bronchitis. The dried

leaves are snuffed to alleviate headaches and fever among other uses (Iwu, 1993).

Anacardium occidentale (Anarcadiaceae) commonly known as cashew is a widely grown plant in South-Eastern Nigeria and it has been reported to possess certain properties which are medicinal (Sokeng *et al.*, 2001). *A. occidentale* leaf is known for its anti-inflammatory effects (Mota et al., 2012). In Brazil, preparations from the bark and leaves are used for the treatment ofeczema, Psoriasis, Scrofula, Dyspepsia, genital problems, and venereal diseases, as well as for impotence, bronchitis, cough, intestinal colic, leishmaniasis and syphilis-related skin disorders (França et al., 2005).

Diarrhoea is the frequent passage of unformed, loose or watery stool, usually three or more times in 24hours (Palombo et al., 2006)**.** There are three clinical types of diarrhea whichinclude acute watery diarrhea (lasts several hours or days, and includes cholera), acute bloody diarrhea (dysentery), andpersistent diarrhoea (lasts 14 days or longer). Clinical syndromes of diarrhea have been defined, each reflecting different pathogenesis and requiring different approaches to treatment. The onset of the disease may be abrupt and self-limiting in immune-competent individuals, but chronic diarrhoea may be persistent even with therapy (WHO, 2017).

In developing countries, diarrhoea continues to be one of the leading causes of mortality in children less than 5 years old. According to World Health Organization (WHO), diarrhoea is the cause of 3.3% of all deaths. More than 5 – 8 million deaths each year in children are accounted for by diarrhea worldwide. The incidence of diarrhoeal diseases remains high despite the effort by many government and international organizations to reduce it (WHO, 2004).

Promising polyherbal formula with impressive preclinical results will warrant a clinical trial. Based on existing claims, this study aims to investigate the combined anti-diarrhoeal effects of aqueous extracts from the leaves of *O. gratissimum* and *A. occidentale* in albino mice.

MATERIALS AND METHODS

Materials

Plant material

Plant part (leaves) of *Ocimumgratissimum* and *Anacardium occidentale*was collected from the plants growing in St. Patrick Catholic Church premise, UwaniAmokwe in Udi L.G.A of Enugu State, Nigeria. The plant materials were authenticated by a Botanist, Dr. Mrs. Bibian Aziagba in Botany department, Nnamdi Azikiwe University Awka, Anambra state, and the voucher numbers (*A.occidentale* = NAU/BOT/291, *O. Gratissimum*= NAU/BOT/315).

Animals

Albino mice of either sex obtained animal house of Veterinary Department of University of Nigeria, Nsukka, weighing 28–38g was used. Animals were between 8–12 weeks old. They are nulliparous and non-pregnant.

Equipments: Hot air oven (Genlab, UK), Electronic weighing balance (Ohaus Corp, USA), water bath (Serological, England), beakers (Pyrex;10ml, 50ml, 100ml, 1000ml), measuring cylinders, Hand grinding machine(Ohaus Corp, USA), Syringes and needles(1ml, 2ml and 10ml capacity), Refrigerator (Thermocool, England), Cotton wool (Pyrex).

Reagents and Chemicals: Concentrated sulphuric acid (Versha Chemicals, Belgaum), naphthol solution in ethanol (Molisch reagents) (Nalco Chemicals, USA), Ammonium solution (Shakti Chemicals, India), Aluminum chloride (Neel Chemicals, India), Fehling solution A and B (Alpha Chemika, India), Hager's reagent (saturated solution of picric acid) (Alpha Chemika, India), Wagner's reagent (iodine and potassium iodide) (Alpha Chemika, India). , Loperamide, Castor oil,

Methods

Preparation of extracts

The fresh leaves of *O. gratissimum* and *A. occidentale* were air-dried at room temperature over 7 weeks, after which they were crushed using a dry mechanical mill. A quantity 150g each of the powder was poured separately into different extraction chambers and macerated with 100ml of distilled water each, and then concentrated in a water bath at a constant temperature of 20^0C. The required quantities of both extracts were mixed and administration in equal ratios (according to herbalists' direction and approval of my supervisor). The extracts before usage were preserved in a refrigerator at 8^0C.

Acute toxicity study

Acute toxicity study was done according to a modified method employed by Lorke, 1983using albino mice on the aqueous leaf extracts of *Ocimum gratissimum* and *Anacardium occidentale*. This method has two phases. In phase 1, groups (n = 3) of animals received oral doses of 10, 100 and 1000 mg/kg body weight of the extract. In the second phase, another different sets of mice were randomized into 3 groups (n = 3) and each group received 2000, 3000, 4000 and 5000 mg/kg of the extract. All animals were monitored for signs of toxicity and mortality. The oral median lethal dose was calculated using the formula: LD50 = √ minimum toxic dose × maximum tolerated dose.

Castor Oil-Induced Diarrhoea

The anti-diarrheoa activity of the extracts was carried out using animal model as was described by (Nwafor and Hamza. 2007). A total of thirty-three adult albino mice of both sexes were used. They were grouped into eleven groups of three mice per group. The animals were fasted

for 18hrs and tested. Group 1 animals served as negative control and were administered distilled water (10ml/kg, P.O). Group 2 animals served as positive control and were administered loperamide (2mg/kg, P.O). Groups 3, 4 and 5 animals were given aqueous extract of *Ocimum gratissimum* at doses (50mg/kg, 200mg/kg and 800mg/kg), respectively. Group 6, 7 and 8 animals were administered aqueous extracts of *Anacardium occidentale* at doses (50mg/kg, 200mg/kg and 800mg/kg, P.O) respectively. Groups 9, 10 and 11 animals received the combined aqueous extracts *Ocimum grastissimum* and *Anacardium occidentale* (ratio: 1:1) at doses (50mg/kg, 200mg/kg and 800mg/kg).

Then 30 minutes post-treatment, diarrhoea was induced by a single oral administration of 0.2ml of castor oil. The animals were observed under glass beakers with the floor lined with filter paper, and the following parameters were noted; onset of diarrhoea, the number, and weight of wet stool was noted 1hr, 2hrs, 3hrs, and 4hrs post diarrhoea induction. The test groups and reference group was compared with the control group for a significant difference.

Gastrointestinal motility study

The effect of the aqueous extracts on intestinal propulsion in adult albino mice was tested using charcoal meal methods (Akter *et al*., 2010). A total of thirty-three adult albino mice of both sexes were used and they were grouped into eleven groups of three animals in each group. The animals were fasted 18 hours and tested, and thena 5% charcoal meal was prepared in the mucilage of tragacanth. Group 1,2 and 3 received an aqueous extract of *Ocimum grastissimum* at doses (50mg/kg, 200mg/kg and 800mg/kg, P.O) and each animal was given 0.5ml of the charcoal meal orally immediately after treatment. Groups 4, 5, and 6 received an aqueous extract of *Anacardium occidentale* at doses (50mg/kg, 200mg/kg and 800mg/kg, P.O), and also received 0.5ml of the charcoal mealimmediately. Groups 7, 8 and 9 received combined aqueous extracts of both extracts (ration 1:1) at doses (50mg/kg, 200mg/kg and 800mg/kg, P.O) and each immediately received 0.5ml of charcoal meal orally after treatment. Group 10 served as positive control and received 10mg/kg atropine I.P and 0.5ml of charcoal meal P.O. Group 11 received distilled water (10mg/kg P.O) and immediately 0.5ml of charcoal meal orally. Then 15 minutes post-treatment, each of the animals was sacrificed by cervical dislocation, dissected and their small intestine layed aside. The mesentery was cut loosed so that the entire length of the intestine was free. The distance travelled by the charcoal plug was measured using meter rule and was expressed as a percentage of the entire length from the pyloric region to the caecum.

Data analysis

Data are presented as mean ± Standard error of the mean (SEM). One-way ANOVA with Dunnett's post-test was performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com $P < 0.05$ was considered to be statistically significant.

RESULT

Acute toxicity

The result of the lethal acute toxicity confirms that the aqueous extracts of *Ocimum gratissimum* and *Anacardium occidentale*, in combined form, when administered, showed no mortality at the dose level up to 5000mg/kg.

Antidiarrhoeal studies

Effects of the aqueous extracts on castor oil-induced diarrhoea

Higher concentrations of the aqueous extracts both singly or in combined form decreasedthe number and weight of wet stool.The combined form of the extract at doses 50mg/kg, 200mg/kg and 800mg/kg showed greater effect compared to the corresponding doses of single extract, and this effect was more pronounced as the time increases. The dose that produced the greatest effect was the combined extracts at a dose of 800mg/kg. At higher concentrations, the time interval beforetheonset of diarrhea was longer but shorter in lower concentrations.

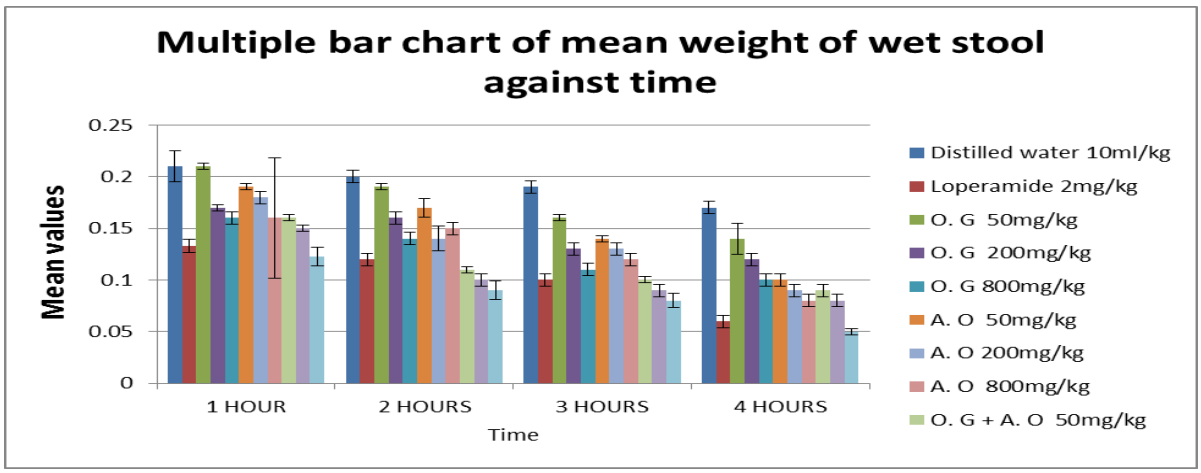

Figure 1: Mean Weight of Wet Stool: (Mean±Sem).

Table 1: Mean Number Of Wet Stool: (Mean±Sem).

GROUP	DOSE	1 HR	2HRS	3HRS	4 HRS
O.G	50mg/kg	4.33 ±0.33	3.00 ± 0.58	3.00 ± 0.58	2.67 ± 0.33
O.G	200mg/kg	4.00 ±0.33	3.00 ± 0.58	3.00 ± 0.58	2.00± 0.58
O.G	800mg/kg	3.67 ±0.33	2.67 ± 0.00	2.00 ± 0.333	2.00 ± 0.00
A.O	50mg/kg	4.00 ± 0.33	3.00 ± 0.58	2.67 ± 0.333	2. 33 ± 0.33
A.O	200mg/kg	3.67 ± 0.33	3.00 ± 0.00	2.00 ± 0.00	2.33 ± 0.33
A.O	800mg/kg	3.33 ± 0.33	2.67 ± 0.33	2.00 ± 0.33	2.00 ± 0.33
O.G + A.O	50mg/kg	3.67 ± 0.00	3.33 ± 0.33	2.33 ± 0.33	2.00 ±0.00
O.G + A.O	200mg/kg	2.33 ± 0.33	2.677 ±0.33	2.00 ± 0.00	2.00 ± 0.00
O.G + A.O	800mg/kg	3.33 ± 0.33	2.67 ± 0.33	2.00 ± 0.33	1.00 ± 0.00
Loperamide	2mg/kg	3.33 ± 0.33	2.33 ± 0.33	1.67 ± 0.33	1.00 ±0.00
Distilled water	10ml/kg	4.33 ± 0.33	3.67 ± 0.33	3.00 ± 0.00	3.00 ± 0.333

Key
O.G = *Ocimum gratissimum*
A.O = *Anacardium occidentals*
SEM = Standard error of mean

Effects of the Aqueous extracts on gastrointestinal motility

In the gastric motility experiment, a similar trend was seen.The combined extract recorded more reductions in gastric motility than the single extracts. Theshortest distance travelled (40%) by the charcoal meal in the gastrointestinal motility was at 800mg/kg combined extract. A significant delay in the onset of defecation ($p<0.05$), reduction in the cumulativefaecal weight ($p<0.001$), along with a change in the faecal consistency from watery to solid form was observed at the dose of 800mg/kg in the castor oil-induced diarrhoea model.

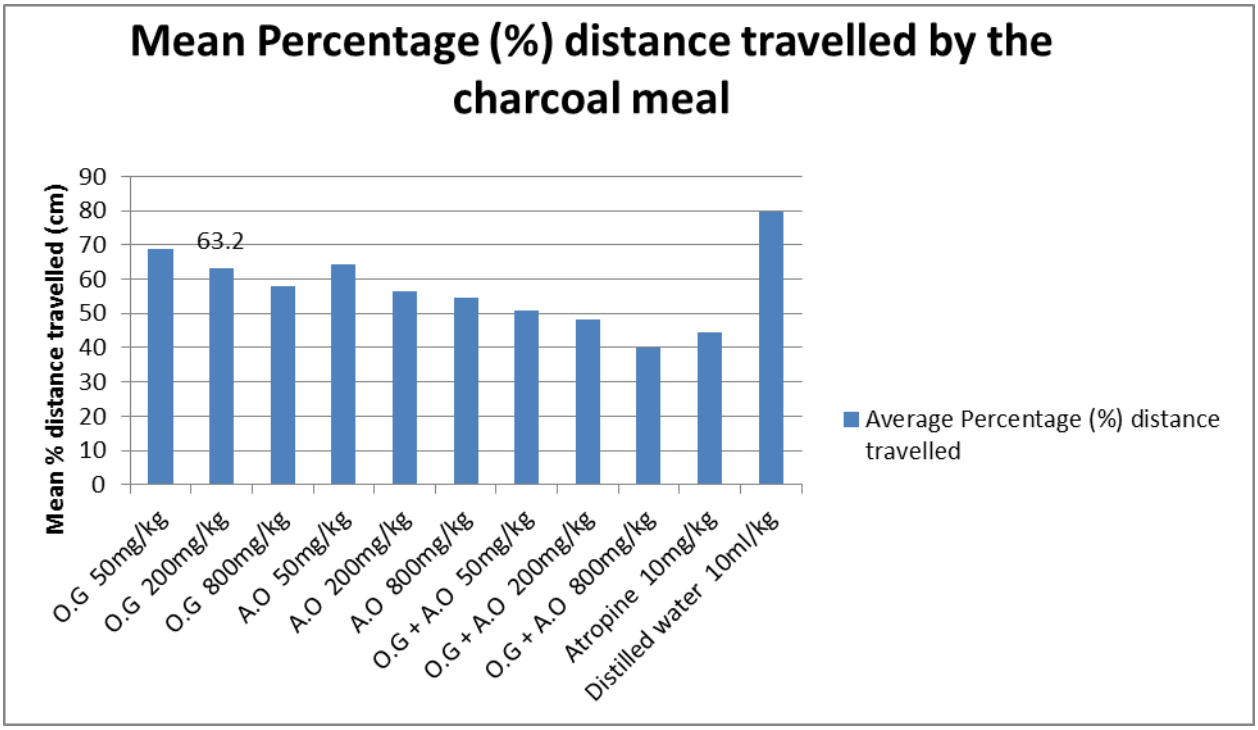

Figure 2: Mean percentage (%) distance travelled by the charcoal meal.

DISCUSSION

As earlier pointed out, a polyherbal anti-diarrhoeal formula has obvious benefits. This present study showed that the LD_{50} of combined aqueous extracts of *Ocimum gratissimum*and *Anacardium occidentale* (1:1) at a dose of 5000 mg/kg orally practically did not cause any death nor any sign of toxicity observed. The combined extract demonstrated a dose and time-dependent activity (Onyegbule *et al*., 2019 and Bruce *et al*., 2016).

The extracts inhibited castor oil-induced diarrhoea in adult albino mice as judged by a decrease in the number of wet faeces in the extract-treated mice. The inhibition was dose and time-dependent since there was a decrease in the number and weight of wet faeces as dose and time

increases. Similarly, the combined form of the extract at doses 50mg/kg, 200mg/kg and 800mg/kg showed greater anti-diarrhoea effect compared to the corresponding doses of single extract, and this effect was more pronounced as the time increases. The dose that produced the greatest anti-diarrhoeal effect was the combined extracts at dose 800mg/kg. The higher theconcentration, the earlierthe onset of diarrhea. Activated charcoal was used in the gastrointestinal motility test to investigate the effects of these extracts on the peristaltic movement. According to the results, the propulsion of charcoal meal are suppressed by these extracts (probably in the same way as atropine sulphate) and results in increased time for absorption of water and electrolytes (Rupa and Abdul, 2015). Inspite of the fact that the concept of polyherbalism is unique to Ayurveda, it is difficult to explain in terms of modern parameters. Historically, the Ayurvedic literature highlights the concept of synergism behind polyherbal formulations. The popularity of single herb formulation isas a result of their active phytoconstituents, which are usually present in minute quantity and are often insufficient for the desired therapeutic effects to be achieved (Sarangdhar, 2015).

Scientific studies have revealed that these plants of varying potency when combined may theoretically produce more potent effectthan individual plants. Thus a positive herb-herb interaction could result insynergism. This synergisticinteractioncould be pharmacokinetic or pharmacodynamic. The popularity of polyherbal formulation is due to their perceivedeffectiveness in a vast number of diseases (Tayade*et al*, 2015). It has been suggested thatthey have a wide therapeutic range (effective at low dose and safe at high dose), fewer side effects, areeco-friendly, cheaper and readily available.This notwithstanding, polyherbal formulations are not always safe(Charaka Samhita, 2013).Therefore, the extracts are thought to inhibit peristaltic movements in a charcoal meal test, and intestinal fluid secretions in castor oil,promotinganti-diarrhoeal activity. This activity could be primarilydue to its high flavonoid and tannin content. The results provide evidence that the aqueous extracts of *O. gratissimum* and *A. occidentale* synergises in anti-diarrhoeal activity.

CONCLUSION

This present study showed that there is synergism in the antidiarrheal properties of the combined aqueous extracts of *O. gratissimum* and *A. occidentale* (1:1), at a high dose of 800mg/kghas the best anti-diarrhoea property.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

1. Akter R, Hasan SR, Hossain MM, Jamila M, Chowdhury SS, Mazumder ME & Rahman S Antidiarrhoea and antioxidant properties of *curcuma alismatifoha* leaves. Australian Journal of Basic and Applied Sciences, 2010; 4(3): 450-456.
2. Ammon PJ, Thomas, Philips S Effects of oleic acid and ricinolic net jejuna water and electrolyte movement. J Clin Invest, 1974; 53: 374-9.
3. Aremu, O., Lawoko, S. Moradi, T., Dalal, K Socio-economic determinants in selecting childhood diarrhoea treatment options in sub-saharan Africa: A multilevel model *Italian j. paed.* 2011; 37: 1-8.
4. BairagiS.M, Aher A.A, Nema N, Pathan I.B Evaluation of Anti-Diarrhoeal Activity of the Leaves Extract of *Ficus Microcarpa* (Moraceae) 138Marmara Pharm J, 2014; 18: 135-138.
5. Bruce SO, Onyegbule FA, Ihekwereme CP Evaluation of the hepato-protective and anti-bacterial activities of ethanol extract of *Picralimanitida*seed and pod. Journal of Phytomedicine and Therapeutic, 2016; 15(2): 1-22.
6. Charaka Samhita Complete encyclopedia of ayurvedic science, int. J. Alt. Med, 2013; 1(1): 12-20.
7. Chorgade MS Drug discovery and Development. Vol. 2.Hoboken, New Jersey; John Wiley and Sons Inc. Drug Development, 2007.
8. Ezeigbo, I. I, Ezeja, M. I, Madubike, K. G, and Ifenkwe, D. C Evaluation of *Anacardium occidentale* Methanol leaf extracts in experimental diarrhoea of mice. Nigerian Veterinary Journal, 2012; 33(4): 624 – 629.
9. França LR, Avelar GF, Almeida FFL Spermatogenesis and sperm transit through the epididymis in mammals with emphasis on pigs. Theriogenology, 2005; 63(2): 300–318.
10. Ilori MO., Sheteolu AO., Omonigbehin EA., and Adeneye AA Anti-diarrhoeal activities of *Ocimum gratissimu* (Lamiaceae). Nigerian Institute of Medical Research, 2013.
11. Iwu MM Handbook of African medicinal plants CRC Press Inc. Boca Raton, Floridamm, 1993.
12. Lorke D A new approach to practical acute toxicity testing. Arch Toxicol, 1983; 54: 275-87.
13. Mota ML, Lobo LT, Costa JM, et al In vitro and in vivo antimalarial activity of essential oils and chemical components from three medicinal plants found in northeastern Brazil. Planta Med, 2012; 78(7): 658– 664.
14. Nwafor PA & Hamza HG Antidiarrhoeal and anti-inflammatory effects of methanolic extract of Guiera senegalenses leaves in rodents. Journal of Natural Remedies, 2007; 7(1): 72-79.
15. Onyegbule FA, Bruce SO, OnyekweON Onyealisi OL, Okoye PC Evaluation of the in vivo antiplasmodial activity of ethanol leaf extract and fractions of *Jatropha gossypifolia*in Plasmodium berghei infected mice. Journal of Medicinal Plants Research, 2019; 13(11): 269-279.
16. Palombo, E. A Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea; modes of action and effects on intestinal function. Phytother. Res., 2006; 20: 717-724.

17. Prabhu K.S, Lobo R, Shirwaikar A.A and Shirwaikar A Ocimum gratissimum: A Review of its Chemical, Pharmacological and Ethnomedicinal Properties. The Open Complementary Medicine Journal, 2009; 1: 1-15.
18. Rupa S, and Abdul B.A Evaluation of antidiarrheal activity of ethanolic extract of Bauhinia variegate (leguminosae) stem bark in wister albino rats. International Journal of Pharmaceutical and Phytopharmacological Research, 2015; 5(1): 2-7.
19. Sokeng, S. D., Kamtchouing, P., Watcho, P., Jasta, H.B., Moundopa, P. F., Lontsi, D. Hypoglycemic activity of *Anacardiumoccidentale* leaves aqueous extract in normal and streptozotocin-induced diabetic rats. *Diabetes Res.*, 2001; 36: 001 – 009.
20. Subramani P, GanSiaw T and Sokkalingam A.D Polyherbal formulation, concept of Ayurveda; Pharmacognosy Review, 2014; 8(16): 73 – 80.
21. Sripad K, Kowalli S, Metri R.B. Serum biochemical and hematological profile of male, female and different age groups of Krishnavalley breed of cattle in Karnataka. International Journal of Pharma and Bio Sciences, 2014; 5(2): B176-B180.
22. Tayade J.A and A V Patil Smt. Sharadchandrika Suresh Patil Abstract on polyherbal formulation journal,College of Pharmacy, India Tripathi KD (2003). Essential of Medical Pharmacology 5th Ed. New Delli. Jaypee Brothers, 2015; 615-623.
23. WHO (2004). Mortality and Burden of Disease Estimates for WHO Member States. *World Health Organization.* WHO Diarrhoeal disease**.** *World Health Organization,* 2017.