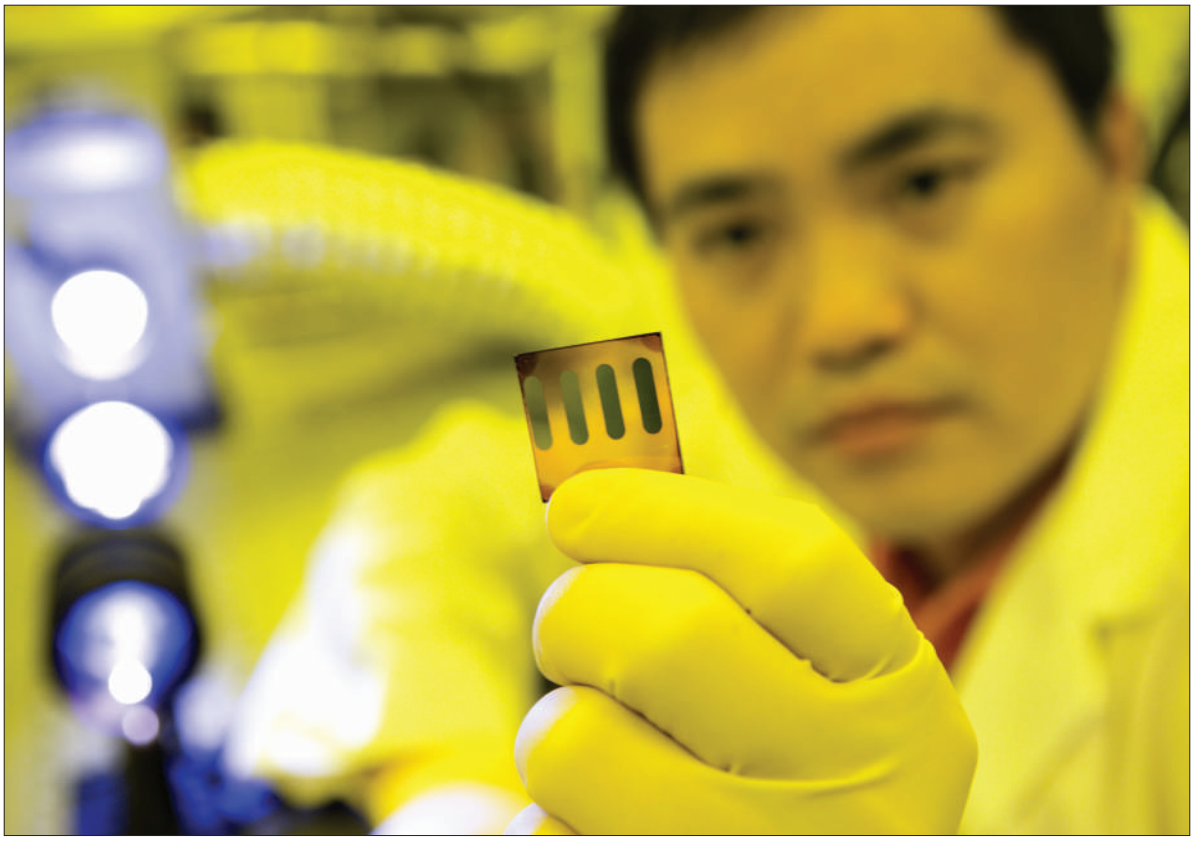

Singapore Journal of

Scientific Research

ISSN: 2010-006x

science alert

http://scialert.net/sjsr

OPEN ACCESS Singapore Journal of Scientific Research

ISSN 2010-006x
DOI: 10.3923/sjsres.2020.94.104

Research Article
In vitro Phytochemical, Antidiarrhoea and GC-MS Screening of the Methanol Leaf Extract of *Cassia siamea* (Fabaceae)

[1]Cyril Ogbiko, [2]Gideon Owoicho Okpanachi and [1]Ikilima Bandi Ibrahim

[1]Department of Pure and Applied Chemistry, Usmanu Danfodiyo University, PMB 2346, Sokoto, Sokoto State, Nigeria
[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Gombe State University, Gombe State, Nigeria

Abstract

Background and Objective: In Nigeria, diarrhea is responsible for the mortality and morbidity of over 2 million children yearly hence there is a need to search for new antidiarrheal agents. The study was aimed to investigate the methanol extract of *Cassia siamea* against experimental diarrhea induced by castor oil. **Materials and Methods:** The oral antidiarrheal effect was evaluated *in vivo* in three groups of rats receiving 200, 400 and 800 mg kg^{-1} b.wt., respectively. Two other groups received 1% Tween-80 (2 mL kg^{-1}) and loperamide (5 mg kg^{-1}) as negative and positive control respectively. The effect of the extract on intestinal transit and intestinal fluid accumulation was assessed. The acute toxicity and the active constituents of the extract were also determined via preliminary phytochemical and GC-MS screening. **Results:** The extract dose dependently delayed the time of appearance of the first stools and decreased the frequency of defecation with respective percentage of 43.33, 51.52 and 58.27%, respectively. The protective role of the extract at 800 mg kg^{-1} was comparable to that of loperamide as well as produced a decrease in intestinal transit comparable to atropine as well as significantly ($p<0.05$) inhibited castor oil-induced enteropooling. The GC-MS analysis confirmed the occurrence of 6 prominent phytocompounds established to possess anti-diarrhoeal effect. No mortality and visible signs of toxicity were observed in the animals following the highest extract administration of 3000 mg kg^{-1}. **Conclusion:** The results showed that the methanol extract of *C. siamea* has a significant antidiarrheal activity devoid of acute toxicity which supports its use in traditional herbal medicine practice.

Key words: *Cassia siamea*, antidiarrheal, castor oil, intestinal transit, enteropooling, acute toxicity

Citation: Cyril Ogbiko, Gideon Owoicho Okpanachi and Ikilima Bandi Ibrahim, 2020. *In vitro* phytochemical, antidiarrhoea and GC-MS screening of the methanol leaf extract of *Cassia siamea* (Fabaceae). Singapore J. Sci. Res., 10: 94.104.

Corresponding Author: Cyril Ogbiko, Department of Pure and Applied Chemistry, Usmanu Danfodiyo University, PMB 2346, Sokoto, Sokoto State, Nigeria
Tel: 08080398933

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diarrhea is an alteration in normal bowel movement occasioned with the passage of daily stools exceeding 300 g and contains 60-95% water[1]. Diarrhea is accompanied with an increase in gastrointestinal motility, secretion as well as a decrease in fluid and electrolytes absorption by the small intestine[2,3]. Diarrhea is caused by four pathophysiologic processes: increased luminal osmolarity, electrolytes secretion, decreased electrolytes absorption and abnormal intestinal motility causing reduction in intestinal transit time[4]. In the intervention of diarrhea, antimotility and antisecretory agents remain as the main agents used to decrease such pathophysiologic changes[5]. It is responsible for about 2-4 million annual infant mortality and morbidity in developing countries especially in Africa[6]. While opioids notably diphenoxylate, difenoxin and loperamide are the drug of choice widely used in the treatment of diarrhea, there are many other drugs that have antimotility or antisecretory effects on the intestine and can be used for the treatment of diarrhea including antimicrobial agents which help to reduce the severity and duration of infectious diarrhea[7]. It is reported that certain medications used to treat other disorders may induce diarrhea for example, theophylline preparations causes diarrhea secondary to alteration of mucosal cyclic adenosine monophosphate (cAMP) as well as reserpine and guanethidine which induces diarrhea by changing gut neuronal input and reducing noradrenergic mediated relaxation[7].

Several rural populations in Nigeria reside far away from primary health centers, thus limiting their access to proper medication. In such scenario, medicinal plants appear as the sole alternative and available health care solution. Medicinal plants have long been explored since antiquity as important source of new drugs as many plant species have been screened for bioactive compounds with therapeutic efficacy. It is in the light of this that the World Health Organization (WHO) encourages research aimed at the prevention and treatment of diarrheal diseases since the current drugs of choice are linked with adverse effects and contraindications[8,9] as well as resistance in the antibiotics used in the treatment of diarrhea[10].

Cassia siamea belongs to the sub-family Fabaceae (Caesalpinioideae) of family Leguminosae[11]. It is widely distributed in various countries including Asia, South Africa, Mexico, China, West Indies, East Africa and Brazil (Fig. 1). It was believed to be introduced to Africa from tropical Asia. The leaves of the plant is used traditionally as vegetables in Thailand[12] as well as ethno-medicinally as laxative, blood cleaning agent, cure for digestive system, urinogenitory disorders, herpes and rhinitis[13], constipation, diabetes, insomnia[14], hypertension, asthma, typhoid fever, diuresis[15], antimalarial agent[16], ringworm and other fungal skin infections[17], dysentery and disorders of the large intestine[18].

Several mechanisms had been previously proposed to explain the diarrheal effect of castor oil. These include inhibition of intestinal Na^+/K^+ ATPase activity, thus reducing normal fluid absorption[19], activation of adenylate cyclase or mucosal cAMP-mediated active secretion[20], stimulation of prostaglandin formation[21] and platelet activating factor[22]. Most recently nitric oxide has been claimed to contribute to the diarrheal effect of castor oil[23]. However, it's ability to induce diarrhoea is due to its most active component ricinoleic acid through a hypersecretory response[24,25]. Vital tools used to assess for new antidiarrheal substances are

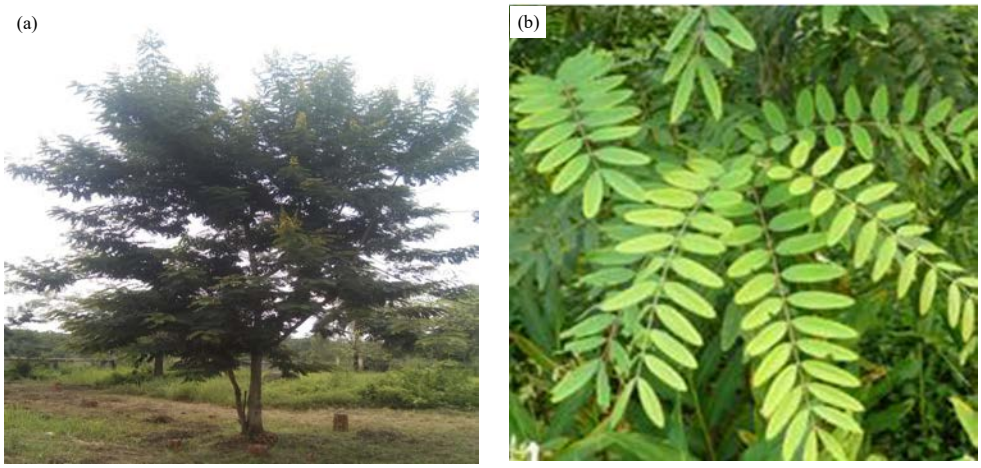

Fig. 1(a-b): *Cassia siamea*, (a) Tree and (b) Leaves in its natural habitat

castor oil-induced diarrhea, activated charcoal meal and isolated intestinal assays respectively[26]. Traditionally, people use plants or plant-derived preparations as remedy for diarrheal disorders without any scientific basis, thus the aim of this study was to evaluate the *in vivo* antidiarrheal activity of the methanol leaf extract of *C. siamea*, its acute toxicity, phytochemical screening and confirmation of its bioactive compounds via GC-MS.

MATERIALS AND METHODS

Drugs and chemicals: The list of drugs employed are methanol, atropine sulphate and loperamide, castor oil, normal saline and charcoal meal (which contain 10% activated charcoal in 100 mL of 5% aqueous gum acacia). Standard reagents were used for the phytochemical screening. All chemicals and reagents were analytical grade.

Collection, preparation and authentication of the plant material: Fresh mature *Cassia siamea* leaves used in this study were collected by Mr. Jude Ibeabuchi Ali from a forest in Owerri, Imo State South-East Nigeria in June, 2018. They were immediately transported fresh to the Department of Pure and Applied Chemistry, Usmanu Danfodiyo University Sokoto, North-Western Nigeria. The plant materials were authenticated at the herbarium unit of the Department of Plant Biology, Usmanu Danfodiyo University, Sokoto where voucher specimen was deposited. The leaves were washed with clean tap water to remove dust and earthy impurities before being air dried for three weeks and pulverized into fine powder using a clean mortar and pestle. The powdered samples were kept in a clean air tight glass container until ready for use.

Preparation of extract: The procedure of Ogbiko[27] with slight modification was adopted. One kilogram of the powdered sample was extracted in a Soxhlet apparatus for 6 h with methanol. The extract was concentrated to dryness under vacuum at a temperature of 45°C by using rotary evaporator (Buchi, Switzerland), dried completely and stored in a clean sterile glass bottle before being preserved in a refrigerator at 4°C until ready for further use. The percentage yield with respect to the original mass extracted was determined.

Preliminary phytochemical screening: The preliminary phytochemical screening using various qualitative chemical tests was performed to detect the presence of various classes of phytoconstituents in the methanol leaf extract of the plant was performed according to the methods outlined by Savithramma *et al.*[28] and Jones and Kinghorn[29] to determine the presence or absence of alkaloids, tannins, flavonoids, saponins, phenols, phytosterols, cardiac glycosides and reducing sugars. These phytoconstituents were identified by characteristic color changes using standard procedures.

Procurement and handling of experimental animals: Healthy Swiss albino mice of both sexes, weighing 22-34 g, were used for the acute toxicity study whereas Wistar albino rats weighing 120-190 g of either sex were used for the antidiarrheal activity. The animals were procured from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Kaduna State Nigeria and transported to the Animal House of the Department of Pharmacology and Therapeutics, Usmanu Danfodiyo University, Sokoto, Sokoto State Nigeria. They were acclimatized to the working laboratory conditions for 14 days by housing them in plastic cages with softwood shavings and chips as beddings. They had free access to clean water and pelletized food *ad libitum*. The equipments, handling and sacrificing of the animals were in accordance with the standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC directives[30].

Grouping and dosing of animals: Twenty mice (for the acute toxicity study) in regardless of sex were randomly divided into 4 groups of 5 mice per group. While groups A, B and C (treatment groups) received 1000, 2000 and 3000 mg kg^{-1} b.wt., of the extract, group D (control) received 1 mL of 1% Tween-80 (the vehicle used for the extract preparation). Seventy five Wistar albino rats (80-200 g) in regardless of sex for the *in vivo* antidiarrhoea, gastrointestinal motility and enteropooling studies were randomly divided into 5 groups of 5 rats/group for each study. In all models, group A (negative control) were treated with the vehicle (1% Tween 80, 2 mL kg^{-1}). The positive controls (group B) received 5 mg kg^{-1} of loperamide (for the castor oil-induced diarrhea and enteropooling models) and atropine 1 mg kg^{-1} (in the gastrointestinal motility test model). The treatment groups (groups C, D and E) in each model received 200, 400 and 800 mg kg^{-1} doses of the crude extract respectively.

Preliminary acute toxicity test: The animals were observed individually for signs of toxicity, intake of food and water, mobility, aggressiveness, sensitivity to pain and noise 4 h post treatment at least once in the first 30 min. Mortality was looked out for during the first 24 h and daily for an additional 13 days for a total of 14 days[31].

Anti-diarrheal activity

***In vivo* castor oil-induced diarrhea:** Diarrhea was induced according to the method described by Teke *et al.*[32] with some modifications. Animals were fasted for 24 h prior to the experiment, but had free access to water. Twenty five rats were divided into groups as earlier outlined. After an hour, all animals received 2 mL of castor oil orally by gavage. The animals were kept in separate metabolic cages with transparent plastic container beneath the cage and lined with Whatman paper of uniform weight to collect faces. The severity of diarrhea was assessed hourly for 6 hours duration. Diarrhea was determined by the presence of fluid material in the stool. At the beginning of each hour old papers were replaced with new ones. The mean stool number and diarrhea stool per group was determined and compared with the control groups. The total score of diarrheal faeces for the negative control group was considered as 100%[33]. The results were expressed as a percentage of inhibition of diarrhea using the expression:

$$\text{Inhibition of diarrhea } (\%) = \frac{A - B}{A} \times 100$$

where, A is mean number of defecation caused by castor oil in the negative group and B is mean number of defecation caused by the drug/extract.

Castor oil-induced enteropooling: The castor oil-induced enteropooling test helps to determine the ability of an extract to prevent the accumulation of fluid. Intraluminal fluid accumulation was determined by the method of Sini *et al.*[34]. Twenty five rats were divided into groups of 5 with five animals/group. One hour before the oral administration of castor oil (2 mL animal^{-1}), all groups were dosed as earlier outlined. Two hours later, the rats were sacrificed and the whole length of the small intestine from the pylorus to the caecum was removed with the intestinal contents collected by milking into a graduated tube and their volume measured.

Gastrointestinal motility test: The rats were divided into 5 groups of 5 animals each as earlier outlined with the procedure as outlined by Pazhani *et al.*[35] adopted with slight modifications. All animals were fasted for 18 h but had free access to water after which all animal groups were dosed as earlier outlined. Thirty minutes later, all animal were orally administered with 1 mL of charcoal meal prepared from 10% activated charcoal in 5% gum acacia. Thirty minutes later, all animals were sacrificed with the distance travelled by the charcoal meal from the pylorus to the caecum measured and expressed as percentage of distance moved using the expression:

$$\text{Intestinal transit } (\%) = \frac{D}{L} \times 100$$

where, D is the distance travelled by charcoal (m) and L is the intestinal length (m).

Gas chromatography-mass spectrometry (GC-MS) analysis: The GC-MS analysis was carried out using GC-MS-QP 2010 Plus Shimadzu system and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: Column Elite-1 fused silica capillary column (30 m×0.25 mm 1 D×µL df, composed of 100% dimethyl polysiloxane). For GC-MS operation, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.99%) was used as the carrier gas at constant flow rate of 1 mL min^{-1} and an injection volume of 2 µL. Split ratio 10:1 injector temperature (250°C) and ion-source temperature of 280°C was used. The oven temperature was programmed from 110°C (Isothermal for 2 min) with an increase of 10°C min^{-1} to 200°C then 5-280°C min^{-1}, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV, a scan interval of 0.5 sec and fragments from 40-550 Da. Total GC running time was 60 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. The mass spectra of the components were matched with the data available in the National Institute of Standards and Technology (NIST) library Ver. 2.0 year 2008 library where the retention time, chemical name and molecular weight of the components of the extract was revealed[27].

Statistical analysis: Results were expressed as Mean±SEM. Statistical differences among the experimental groups were assessed by one-way analysis of variance (ANOVA), followed by Dunnett's t-test. 95% confidence interval ($p<0.05$) was considered statistically significant.

RESULTS

Percentage yield of the crude methanol stem bark extract: About 56.25 g corresponding to a percentage yield of 3.75% was obtained for the extraction of the pulverised leaves of *C. siamea*.

Phytochemical screening: The preliminary phytochemical screening of the methanol leaf extract of *C. siamea* showed the presence of important secondary metabolites which is presented in Table 1.

Preliminary acute toxicity test: The result of the toxicity screening showed that the oral administration of the methanol leaf extract of *C. siamea* administered to mice is safe up to a dose of 3000 mg kg^{-1} since they neither showed mortality nor any apparent signs of toxic effect in the animals. This validates the safety of the plant in its use in ethnomedicine.

Effect of *C. siamea* methanol leaf extract on castor oil-induced diarrhea: The extract of *C. siamea* produced a promising antidiarrheal effect in the rats as shown in Table 2.

At doses of 200, 400 and 800 mg kg^{-1} b.wt., the extract significantly decreased ($p<0.05$) the total number of wet faeces produced after the administration of castor oil when compared to the control group. The effect of the highest dose (800 mg kg^{-1} b.wt.) of the extract was lower but comparable to that of the standard drug loperamide at the dose of 5 mg kg^{-1} b.wt.

Effect of *C. siamea* methanol leaf extract on castor oil-induced enteropooling: *Cassia siamea* methanol leaf extract significantly ($p<0.05$) inhibited castor oil-induced enteropooling in rats at oral experimental doses of 200, 400 and 800 mg kg^{-1} b.wt., as presented in Table 3.

Effect of *C. siamea* methanol leaf extract on the intestinal transit of charcoal meal: The result of the intestinal transit of charcoal meal when challenged with the methanol leaf extract of *C. siamea* (Table 4) showed a dose dependent reduction in the progression of the administered charcoal meal in the

Table 1: Phytochemical constituents of the stem bark extract

Phytochemicals	Inference
Alkaloids	+
Flavonoid	+
Glycosides	+
Reducing sugar	+
Saponins	-
Steroids	+
Phenols	+
Tannin	+

+ : Presence of phytochemical, - : Absence of phytochemical

Table 2: Effect of the methanol leaf extract of *C. siamea* (MLECS) on castor-oil induced diarrhea in rat

Treatments	Dose	Total number of faeces	Number of diarrhoea faeces	Inhibition of diarrhoea (%)
1% Tween 80	2 mL kg^{-1} + 2 mL castor oil	24.41±0.42	19.41±0.13	-
Loperamide	5 mg kg^{-1} + 2 mL castor oil	9.44±1.10[a]	4.11±1.00[a]	78.82
MLECS	200 mg kg^{-1} + 2 mL castor oil	18.54±0.11[a]	11.00±0.33[a]	43.33
MLECS	400 mg kg^{-1} + 2 mL castor oil	14.74±0.14[a]	9.41±0.20[a]	51.52
MLECS	800 mg kg^{-1} + 2 mL castor oil	11.22±0.34[a]	8.10±0.10[a]	58.27

MLECS: Methanol leaf extract of *Cassia siamea*, values are expressed as Mean±SEM (n = 5), [a]$p<0.05$ statistically significant relative to the negative control group

Table 3: Effect of the methanol leaf extract of *C. siamea* (MLECS) on castor-oil induced enteropooling in rat

Treatments	Dose	Volume of intestinal fluid (mL)	Inhibition (%)
1% Tween 80	2 mL kg^{-1} + 2 mL castor oil	3.12±0.43	-
Loperamide	5 mg kg^{-1} + 2 mL castor oil	1.10±0.20*	64.74
MLECS	200 mg kg^{-1} + 2 mL castor oil	2.21±0.11	29.17
MLECS	400 mg kg^{-1} + 2 mL castor oil	1.52±0.22*	51.28
MLECS	800 mg kg^{-1} + 2 mL castor oil	1.41±0.04*	54.81

MLECS: Methanol leaf extract of *Cassia siamea*, values are expressed as Mean±SEM (n = 5), *$p<0.05$ statistically significant relative to the negative control group

Table 4: Effect of the methanol leaf extract of *C. siamea* leaf (MLECS) on gastro-intestinal motility

Treatments	Dose	Distance travelled by charcoal (cm)	Intestinal transit (%)
1% Tween 80	2 mL kg^{-1}	81.33±1.13	-
Atropine	1 mg kg^{-1}	51.10±1.03**	56.16
MLECS	200 mg kg^{-1}	61.31±1.22**	67.38
MLECS	400 mg kg^{-1}	59.11±1.00**	64.96
MLECS	800 mg kg^{-1}	53.22±1.09**	58.49

MLECS: Methanol leaf extract of *Cassia siamea*, values are expressed as Mean±SEM (n = 5), **$p<0.05$ statistically significant relative to the negative control group

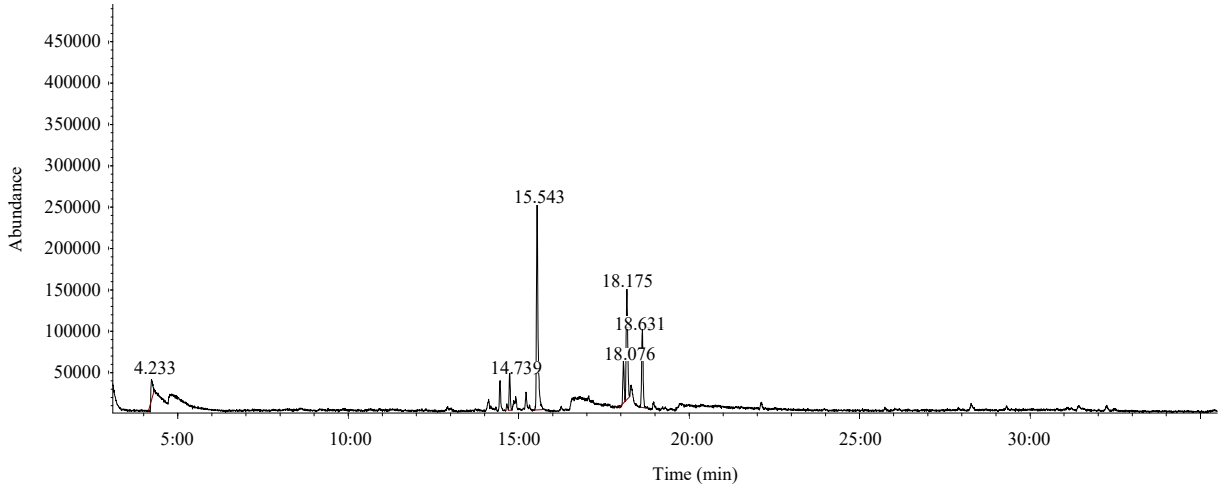

Fig. 2: Gas ion chromatogram of the methanol leaf extract of *C. siamea*

gastro-intestinal tract and hence a reduction in the percentage of the intestinal motility. This activity is significant ($p<0.05$) in all the treatment doses and positive control when compared to the negative control.

GC-MS profiling of the methanol leaf extract of *C. siamea*: The GC-MS profiling of the methanol leaf extract of *C. siamea* revealed the occurrence of 6 peaks corresponding to the presence of 6 prominent chemical compounds as presented in Fig. 2. The mass spectra with the structure of the identified compounds are represented in Fig. 3a-f, respectively.

DISCUSSION

The obtained result of the percentage yield was 3.75% showing that methanol being polar may not be the best solvent for the maximum extraction of the plant phytoconstituents. This differ considerably from the percentage yield of 35 and 33.13% reported by Kamagaté *et al.*[36] and Mehta *et al.*[37], respectively. This may be attributed to factors like extraction method and time as reported by Mehta *et al.*[37] who reported an increase in the percentage extractive yield from 15.92-22.00% when *C. siamea* leaves were extracted for 12-24 h duration. Others related factors could be difference in ecotype, chemotype, phenophases, variations in environment conditions such as temperature, relative humidity, irradiance and photoperiod as well as genetic background off of which affect secondary metabolites of plants[38].

Preliminary phytochemical screening revealed the presence of important secondary metabolites (Table 1) some of which has been established to possess antidiarrhoeal activity. Reports from literature have shown that tannins, flavonoids, alkaloids, saponins, reducing sugar, sterols and/or terpenes[21,39-41] possesses antidiarrhoea activity all of which were found in the methanol extract except saponins which was found to be absent. While tannins have been reported to have an antispasmodic and muscle relaxant effect, flavonoids inhibit prostaglandin E2-induced intestinal secretion[42-44], flavonoids has the ability to inhibit intestinal motility and hydro-electrolytic secretions induced by prostaglandins[45-47] E2 hence could be said to play a role in prostaglandins biosynthesis considered to delay castor oil-induced diarrhea[48]. Polyphenols on the other hand possess antidiarrheal property by interacting and inhibiting cytochrome P450 systems[49].

As shown in Table 2, the plant extract significantly ($p<0.05$) reduced the total fecal output and diarrheal drops in rats in the 6 h observation period compared with the negative control. None of the animals treated with the plant extract showed diarrhoea up to at least an hour after administration of the extract. There was an increasing pattern of diarrheal inhibition with increasing dose of the extract as both the total number of fecal output instances and diarrheal episodes with solid consistency as well as a significant delay in the onset of diarrhea was observed. The antidiarrheal activity of the extract noticeable the highest experimental dose of 800 mg kg^{-1} b.wt., was found to be lower (58.27%) but comparable to the antimotility drug loperamide at the dose of 5 mg kg^{-1} b.wt., (78.82%) which among others is known to decreases intestinal fluid and electrolyte loss as well as provide a rapid and sustained inhibition of the peristaltic reflux through depression of longitudinal and circular muscle activity[1].

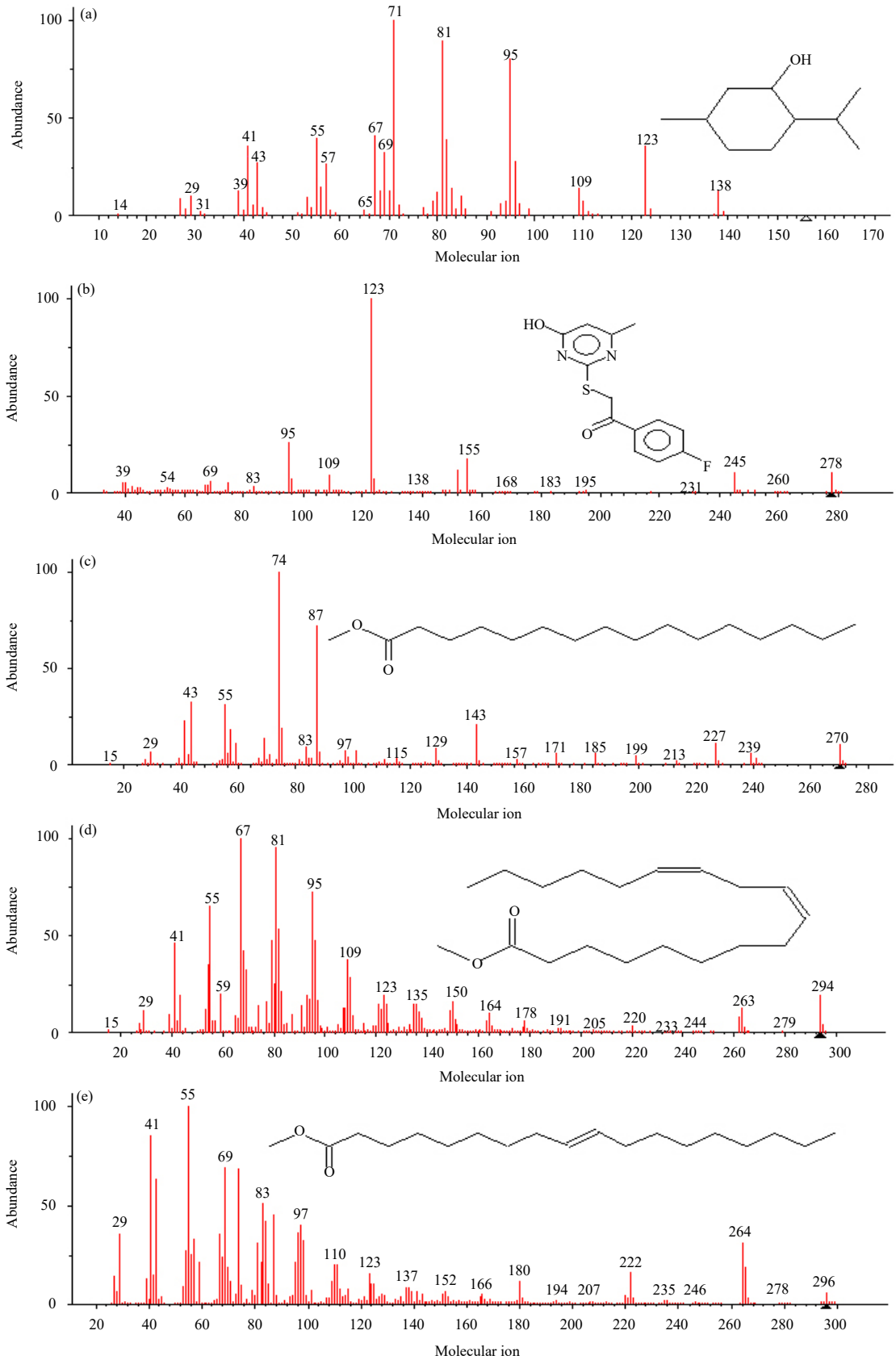

Fig. 3(a-f): Continued

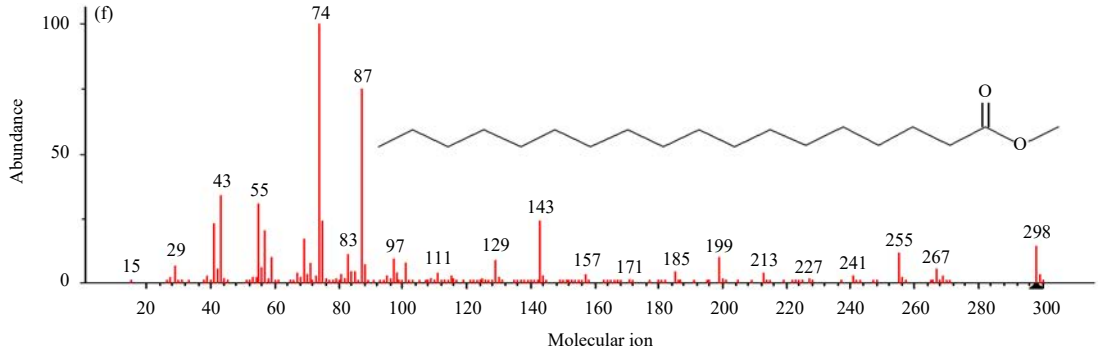

Fig. 3(a-f): Mass spectrum of (a) Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1α,2β,5α)-(+/-.)- [retention time 4.233], (b) 1-(4-Fluorophenyl)-2-[(4-hydroxy-6-methylpyrimidin-2-yl)thio] ethan-1-one [retention time 14.737], (c) Hexadecanoic acid, methyl ester [retention time 15.543], (d) 9,12-Octadecadienoic acid (Z,Z)-methyl ester [retention time 18.076], (e) 9-Octadecadienoic acid, methyl ester [retention time 18.175] and (f) Methyl stearate [retention time 18.631]

In the castor oil induced enteropooling assay (Table 3) all doses of the plant extract showed a significant reduction except the 200 mg kg^{-1} b.wt., of the extract in both the mean weight and the volume of intestinal fluid compared with the negative control.

The inhibition in the volume of intestinal content was observed to increase in a dose dependent manner. The highest dose of the extract on the intestinal fluid accumulation was found to be lower but comparable to the inhibitory effect of the standard antimotility drug loperamide. This shows that the plant extract has a significant antisecretory effect by decreasing water and electrolyte secretion to the intestinal lumen and allowing for their enhanced absorption. This will lead to a decrease in the water contents of the fecal drops hence an overall reduction in the total number of defecation instances and diarrheal drops as observed in the extract treatment groups and positive control group when compared to the negative control group. These actions mimic the mechanism of action of loperamide[50,51].

The decrease in gastrointestinal motility with time allows for the gastrointestinal contents to stay longer in the intestine allowing for enhanced intestinal water and electrolyte absorption. In the gastrointestinal motility investigation (Table 4), the methanol leaf extract of *C. siamea* reduced gastrointestinal motility as shown by the reduction in the gastrointestinal propulsion of the charcoal meal in a dose dependent manner comparable to the standard drug atropine at 1 mg kg^{-1} b.wt., which was statistically significant when compared to the negative control. Several drugs and natural products have been reported to induce their antidiarrheal effect through antispasmodic activity[52]. The extract may hence possess anticholinergic activity which is consistent with the action of atropine known to reduce intestinal motility[39,53,54].

Upon GC-MS analysis, the methanol extract was found to contain 6 prominent constituents eluted between 5-19 min. The compounds and their percentage composition are cyclohexanol-5-methyl-2-(1-methylethyl)-,(1α,2β,5α)-(+/-.)- (3.598%), 1-(4-fluorophenyl)-2-[(4-hydroxy-6-methylpyrimidin-2-yl)-thio]-ethan-1-one (5.848%), hexadecanoic acid methyl ester (44.329%), 9,12-octadecadienoic acid (Z,Z)-methyl ester (6.843%), 9-octadecadienoic acid methyl ester (23.687%) and methyl stearate (15.696%).

These phytoconstituents has been known to have diverse biological activities which have direct bearing to its observed antidiarrheal activity. Cyclohexanol-5-methyl-2-(1-methylethyl), a cyclohexanol analog is known for their antimicrobial activities[55], 1-(4-fluorophenyl)-2-[(4-hydroxy-6-methylpyrimidin-2-yl)-thio]-ethan-1-one a pyrimidine derivative possesses anti microbial and anti-inflammatory activity[56-60], hexadecanoic acid methyl ester a fatty acid methyl ester is a known anti-inflammatory agent[61], 9,12-octadecadienoic acid (Z,Z)-methyl ester, 9-octadecadienoic acid methyl ester and fatty acid methyl ester are known for their antibacterial and antifungal activities[62,63]. Anti-inflammatory compounds are key in diarrheal management since they stimulate intestinal hypersecretion of fluid involved in inflammatory response[63-64].

CONCLUSION

The methanol leaf extract of *C. siamea* reduces castor oil-induced diarrheal episodes, intestinal secretion and motility comparable to loperamide at the highest investigated dose of 800 mg kg^{-1} b.wt. The presence of secondary metabolites with anti-inflammatory and antimicrobial activities provides a potential source for the industrial application of this plant in the development of antidiarrheal drug. These results give scientific credence to the ethnomedicinal application of the plant as an antidiarrheal agent.

SIGNIFICANCE STATEMENT

This study discovers the possible antidiarrheal ability of the methanol extract of *C. siamea* that can be beneficial for the development of novel antidiarrhea agents. This study will help the researchers to uncover the critical area of the pathophysiologic processes responsible for causing diarrhea. Thus, a new theory on the normalization of bowel movement and possibly other contributory factors may be arrived at.

ACKNOWLEDGMENT

The author would like to acknowledge the technical assistance offered by staff of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Sokoto State Nigeria.

REFERENCES

1. Wansi, S.L., E.P. Nguelefack-Mbuyo, M.L. Nchouwet, D. Miaffo and P. Nyadjeu *et al*., 2014. Antidiarrheal activity of aqueous extract of the stem bark of *Sapium ellipticum* (Euphorbiaceae). Trop. J. Pharm. Res., 13: 929-935.
2. Ezeja, I.M., I.I. Ezeigbo, K.G. Madubuike, N.E. Udeh, I.A. Ukweni, S.C. Akomas and D.C. Ifenkwe, 2012. Antidiarrheal activity of *Pterocarpus erinaceus* methanol leaf extract in experimentally-induced diarrhea. Asian Pac. J. Trop. Med., 5: 147-150.
3. Sarin, R.V. and P.A. Bafna 2012. Herbal antidiarrheals: A review. Int. J. Res. Pharm. Biomed. Sci., 3: 637-649.
4. Agbor, G.A., T. Leopold and N.Y. Jeanne, 2004. The antidiarrhoeal activity of *Alchornea cordifolia* leaf extract. Phytother. Res., 18: 873-876.
5. McQuaid, K.R., 2012. Drugs Used in the Treatment of Gastrointestinal Diseases. In: Basic and Clinical Pharmacology, Katzung, B.G., S.B. Masters and A.J. Trevor (Eds.)., McGraw-Hill, New York, USA., pp: 1081-1115.
6. Abdullahi, A.L., M.O. Agho, S. Amos, K.S. Gamaniel and C. Watanabe, 2001. Antidiarrhoeal activity of the aqueous extract of *Terminalia avicennoides* roots. Phytother. Res., 15: 431-434.
7. Mekonnen, B., A.B. Asrie and Z.B. Wubneh, 2018. Antidiarrheal activity of 80% methanolic leaf extract of *Justicia schimperiana*. Evidence-Based Complement. Altern. Med., Vol. 2018. 10.1155/2018/3037120.
8. FDA., 2016. FDA warns about serious heart problems with high doses of the antidiarrheal medicine loperamide (Imodium), including from abuse and misuse. https://www.fda.gov/
9. Anonymous, 2016. Antidiarrheal, drug actions, adverse reactions and contraindication, 2016. https://medipub.blogspot.com
10. Alam, S. and S. Bhatnagar, 2006. Current status of anti-diarrheal and anti-secretory drugs in the management of acute childhood diarrhea. Indian J. Pediat., 73: 693-696.
11. Fowler, D.G., 2006. Traditional fever remedies: A list of Zambian plants. pp: 1-61. http://www.giftsofhealth.org/ritam/news/Traditional_Fever_remedie1.pdf
12. Otimenyin, S.O., J.A. Kolawole and M. Nwosu, 2010. Pharmacological basis for the continual use of the root of *Senna siamea* in traditional medicine. Int. J. Pharma Bio Sci., Vol. 1, No. 3.
13. Aliyu, B.S., 2006. West African Ethnomedicinal Plants. Triumph Publishing Company, Kano, Nigeria.
14. Tripathi, A.K. and K.R. Gupta, 1991. Phytochemical study of *Cassia siamea*. J. Indian Chem. Soc., 68: 254-255.
15. Hill, A.R., 1992. Medicinal plants and traditional medicine in Africa. J. Integr. Med., 39: 42-45.
16. Lose, G.A., S.J. Benard and D.E. Leihner, 2000. Studies on agro forestry hedgerow system with *Senna siamea* rooting patterns and competition effects. J. Sci., 38: 57-60.
17. Rajan, S., D.S. Baburaj, M. Sethuraman and S. Parimala, 2001. Stem and stem bark used medicinally by the Tribals Irulas and Paniyas of Nilgiri district, Tamilnadu. J. Nat. Remedies, 1: 49-54.
18. Hassain, M.K., 1999. *Senna siamea*-a widely used legume tree. Forest, Farm and Community Tree Network (FACT Net) Winrock International, Fact Sheet 99-04, June 1999.
19. Ganinella, T.S. and P. Bass, 1978. Laxatives: An update on mechanism of action. Life Sci., 23: 1001-1009.
20. Capasso, F., N. Mascolo, A.A. Izzo and T.S. Gaginella, 1994. Dissociation of castor oil-induced diarrhoea and intestinal mucosal injury in rat: Effect of N^G-nitro-L-arginine methyl ester. Br. J. Pharmacol., 113: 1127-1130.
21. Galvez, J., A. Zarzuelo, M.E. Crespo, M.D. Lorente, M.A. Ocete and J. Jimenez, 1993. Antidiarrhoeic activity of *Euphorbia hirta* extract and isolation of an active flavonoid constituent. Planta Medica, 59: 333-336.

22. Pinto, A., G. Autore, N. Mascolo, R. Sorrentino, A. Biondi, A.A. Izzo and F. Capasso, 1992. Time course of PAF formation by gastrointestinal tissue in rats after castor oil challenge. J. Pharm. Pharmacol., 44: 224-226.
23. Mascolo, N., A.A. Izzo, T.S. Gaginella and F. Capasso, 1996. Relationship between nitric oxide and platelet-activating factor in castor-oil induced mucosal injury in the rat duodenum. Naunyn-Schmiedeberg's Arch. Pharmacol., 353: 680-684.
24. Ammon, H.V., P.J. Thomas and S.F. Phillips, 1974. Effects of oleic and ricinoleic acids on net jejunal water and electrolyte movement. Perfusion studies in man. J. Clin. Investig., 53: 374-379.
25. Gaginella, T.S., J.J. Stewart, W.A. Olsen and P. Bass, 1975. Actions of ricinoleic acid and structurally related fatty acids on the gastrointestinal tract. II. Effects on water and electrolyte absorption *in vitro*. J. Pharmacol. Exp. Ther., 195: 355-361.
26. Li, J., X.L. Wu, Y. Chen, Z. Tang and Y.H. Xu *et al*., 2013. Antidiarrheal properties of different extracts of Chinese herbal medicine formula Bao-Xie-Ning. J. Integr. Med., 11: 125-134.
27. Ogbiko, C., 2020. Phytochemical, GC-MS analysis and antimicrobial activity of the methanol stem bark extract of *Cassia siamea* (Fabaceae). Asian J. Biotechnol., 12: 9-15.
28. Savithramma, N., M.L. Rao and D. Suhrulatha, 2011. Screening of medicinal plants for secondary metabolites. Middle-East J. Sci. Res., 8: 579-584.
29. Jones, W.P. and A.D. Kinghorn, 2012. Extraction of Plant Secondary Metabolites. In: Natural Products Isolation, Sarker, S. and L. Nahar (Eds.)., Methods in Molecular Biology (Methods and Protocols), Vol. 864. Humana Press, USA., pp: 341-366.
30. EEC., 1986. Council directive 86/609/EEC, of 24 November 1986, on the approximation of laws, regulations and administrative provisions of the member states regarding the protection of animals used for experimental and other scientific purposes. Off. J. Eur. Commun., L358: 1-28.
31. Ogbiko, C., J.C. Eboka, I. Igbe and D.M. Usman, 2017. Anti-Ulcer activity of methanol extract of *Plantago rugelii* Decne. (Plantaginaceae). Trop. J. Nat. Prod. Res., 1: 84-88.
32. Teke, G.N., J.R. Kuiate, O.B. Ngouateu and D. Gatsing, 2007. Antidiarrhoeal and antimicrobial activities of *Emilia coccinea* (Sims) G. Don extracts. J. Ethnopharmacol., 112: 278-283.
33. Abdullahi, M., G. Muhammad and N.U. Abdulkadir, 2003. Combretaceae: Medicinal and Economic Plants of Nupeland. Jube Evans Books and Publications, Bida, Nigeria, pp: 68.
34. Sini, J.M., I.A. Umar, K.M. Anigo, I. Stantcheva, E.N. Bage and R. Mohammed, 2008. Antidiarrhoeal activity of aqueous extract of *Combretum sericeum* roots in rats. Afr. J. Biotechnol., 7: 3134-3137.
35. Pazhani, G.P., N. Subramanian, G. Arunchalam, S. Hemalatha and V. Ravichandran, 2001. Antidiarrhoeal potential of *Elephantopus scaber* Linn leaf extract. Indian Drugs, 38: 269-271.
36. Kamagaté, M., C. Koffi, N.M. Kouamé, A. Akoubet, N. Alain, R. Yao and H.M. Die, 2014. Ethnobotany, phytochemistry, pharmacology and toxicology profiles of *Cassia siamea* Lam. J. Phytopharmacol., 3: 57-76.
37. Mehta, J.P., P.H. Parmar and S.H. Vadia, 2014. Separation of phytochemicals from leaf of *Cassia siamea* and their antimicrobial assay. J. Phytochem., 12: 290-302.
38. Marotti, M., R. Piccaglia, E. Giovanelli, S.G. Deans and E. Eaglesham, 1994. Effects of planting time and mineral fertilization on peppermint (*Mentha x Piperita* L.) essential oil composition and its biological activity. Flavour Fragance J., 9: 125-129.
39. Otshudi, A.L., A. Vercruysse and A. Foriers, 2000. Contribution to the ethnobotanical, phytochemical and pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhoea in Lomela area, Democratic Republic of Congo (DRC). J. Ethnopharmacol., 71: 411-423.
40. Venkatesan, N., V. Thiyagarajan, S. Narayanan, A. Arul and S. Raja *et al*., 2005. Anti-diarrhoeal potential of *Asparagus racemosus* wild root extracts in laboratory animals. J. Pharm. Pharm. Sci., 8: 39-45.
41. Galvez, J., A. Zarzuelo, M.E. Crespo, M.P. Utrilla, J. Jiménez, C. Spiessens and P. de Witte, 1991. Antidiarrhoeic activity of *Sclerocarya birrea* bark extract and its active tannin constituent in rats. Phytother. Res., 5: 276-278.
42. Al-Harbi, B.K. and I.M. El-Ashmawy, 2015. The antidiarrheal activity and phytoconstituents of some methanol extracts from *Asteraceae* family. Merit Res. J. Med. Med. Sci., 3: 347-352.
43. Tiwari, P., B. Kumar, M. Kaur, G. Kaur and H. Kaur, 2011. Phytochemical screening and extraction: A review. Internationale Pharmaceutica Sciencia, 1: 98-106.
44. Dosso, K., B.B. N'guessan, A.P. Bidie, B.N. Gnangoran and S. Meite *et al*., 2012. Antidiarrhoeal activity of an ethanol extract of the stem bark of *Piliostigma reticulatum* (Caesalpiniaceae) in rats. Afr. J. Trad. Complement. Alternat. Med., 9: 242-249.
45. Di Carlo, G., G. Autore, A.A. Izzo, P. Maiolino and N. Mascolo *et al*., 1993. Inhibition of intestinal motility and secretion by flavonoids in mice and Rats: Structure-activity relationships. J. Pharm. Pharmacol., 45: 1054-1059.
46. Rao, V.S.N., F.A. Santos, T.T. Sobreira, M.F. Souza, C.L. Melo and E.A. Silveira, 1997. Investigations on the gastroprotective and antidiarrhoeal properties of ternatin, a tetramethoxyflavone from *Egletes viscosa*. Planta Medica, 63: 146-149.
47. De Medina, L.H.F.S., J. Galvez, M. Gonzalez, A. Zarzuelo and K.E. Barrett, 1997. Effects of quercetin on epithelial chloride secretion. Life Sci., 61: 2049-2055.

48. Brijesh, S., P. Daswani, P. Tetali, N. Antia and T. Birdi, 2009. Studies on the antidiarrhoeal activity of *Aegle marmelos* unripe fruit: Validating its traditional usage. BMC Complement. Altern. Med., Vol. 9. 10.1186/1472-6882-9-47.
49. Anderson, J.E., C.M. Goetz, J.L. McLaughlin and M. Suffness, 1991. A blind comparison of simple bench-top bioassays and human tumour cell cytotoxicities as antitumor prescreens. Phytochem. Anal., 2: 107-111.
50. Shah, S., 2004. Evaluation of diarrhea: The challenge continues! Part-I. Indian J. Med. Sci., 58: 75-78.
51. Chitme, H.R., R. Chandra and S. Kaushik, 2004. Studies on anti-diarrhoeal activity of *Calotropis gigantean* R. Br. in experimental animals. J. Pharm. Pharm. Sci., 7: 70-75.
52. Najeeb-ur-Rehman, M.H. Mehmood, K.M. Alkharfy and A.H. Gilani, 2012. Studies on antidiarrheal and antispasmodic activities of *Lepidium sativum* crude extract in rats. Phytother. Res., 26: 136-141.
53. Bardal, S., J. Waechter and D. Martin, 2010. Gastroenterology. In: Applied Pharmacology, Bardal, S., J. Waechter and D. Martin (Eds.)., Saunders, Pennsylvania, USA., pp: 177-188.
54. Brown, J.H. and P. Taylor, 1996. Muscarinic Receptor Agonists and Antagonist. In: Goodman and Gilman, the Pharmacological Basis of Therapeutics, 9th Edn., Hardman, J.G. and L.E. Limbird (Eds.)., McGraw Hill, New York, pp: 148-154.
55. Mahyavanshi, V., S.I. Marjadi and R. Yadav, 2017. Synthesis and pharmacological studies of 1-(2-amino-1-(4-methoxyphenyl) ethyl) cyclohexanol analogs as potential microbial agents. Arabian J. Chem., 10: S804-S813.
56. Klein, E., S. DeBonis, B. Thiede, D.A. Skoufias, F. Kozielski and L. Lebeau, 2007. New chemical tools for investigating human mitotic kinesin Eg5. Bioorg. Med. Chem., 15: 6474-6488.
57. Bhat, A.R., 2017. Biological activity of pyrimidine derivatives: A review. Organic Med. Chem. Int. J., 2: 1-4.
58. Karale, B.K., C.H. Gill, M. Khan, V.P. Chavan, A.S. Mane and M.S. Shingare, 2002. Synthesis of 3-methyl-4-[(chromon-3-yl) methylene]-1-phenylpyrazolin-5 (4H)-ones and their conversion into 2-thio-5-hydroxy-5H [1]-benzopyrano [4, 3-d] pyrimidines. Indian J. Chem., B41: 1957-1959.
59. Meets, S. and S. Nadeem, 2016. A review on biological importance of pyrimidines in the new era. Int. J. Pharm. Pharm. Sci., 8: 8-21.
60. Saad, H.A. and S.H. Abdel-Hafez, 2012. Synthesis and biological activity of some nucleoside analogs of 3-cyanopyridin-2-one. Curr. Organic Synth., 9: 413-426.
61. Shettima, A.Y., Y. Karumi, O.A. Sodipo, H. Usman and M.A. Tijjani, 2013. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of bioactive components of ethyl acetate root extract of *Guiera senegalensis* J.F. Gmel. J. Applied Pharm. Sci., 3: 146-150.
62. Tyagi, T. and M. Agarwal, 2017. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms. J. Pharmacogn. Phytochem., 6: 195-206.
63. Izzo, A.A., T.S. Gaginella, N. Mascolo and F. Capasso, 1998. Recent findings on the mode of action of laxatives: The role of platelet activating factor and nitric oxide. Trends Pharmacol. Sci., 19: 403-405.
64. Guo, Y.J., T. Luo, F. Wu, Y.W. Mei and J. Peng *et al*., 2015. Involvement of TLR2 and TLR9 in the anti-inflammatory effects of chlorogenic acid in HSV-1-infected microglia. Life Sci., 127: 12-18.