Innovare Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 7, Issue 1, 2015

Original Article

ANTIMICROBIAL AND ANTIDIARRHEAL EFFECTS OF FOUR CAMEROON MEDICINAL PLANTS: *DICHROCEPHALA INTEGRIFOLIA*, *DIOSCOREA PREUSII*, *MELENIS MINUTIFLORA*, AND *TRICALYSIA OKELENSIS*

KAMGANG RENE[1,2]*, FANKEM GAËTAN OLIVIER[2], NGO TETKA JACQUELINE[2], GONSU KAMGA HORTENSE[3], FONKOUA MARIE CHRISTINE[4]

[1]Laboratory of Endocrinology and Radio-Isotopes, Institute of Medical Research and Medicinal Plants Studies (IMPM), Yaoundé, Cameroon, [2]Animal Physiology Laboratory, Faculty of Science, University of Yaoundé I, Cameroon, [3]Laboratory of Bacteriology of YUHC, Cameroon, [4]Laboratory of Bacteriology, Centre Pasteur of Cameroon.
Email: gemskruy@yahoo.fr

Received: 16 Oct 2014, Revised and Accepted: 20 Nov 2014

ABSTRACT

Objective: In order to verify the antidiarrheal activities of *Dichrocephala integrifolia, Dioscorea preusii, Melenis minutiflora, Tricalysia okelensis*, the *in vitro* antimicrobial effect on *Salmonella typhi*, *Escherichia coli*, *Shigella dysenteriae* A_1 and *Candida albicans*, and the *in vivo* antidiarrheal activities on the intestine transit of the hydro/ethanol (v/v) plants extracts were studied.

Methods: The antimicrobial effect of the extracts was assayed *in vitro* by the disc diffusion and the agar dilution methods. For *in vivo* study, male and female mice received *per os* castor oil and one hour later different doses of the extracts.

Results: *In vitro*, *D. integrifolia, D. preusii, M. minutiflora,* and *T. okelensis* extract showed concentration-dependent activity against all the tested microbial strains with the inhibition zone ranged from 08 to 24 mm. *D. integrifolia* 0.5 mg/mL showed the lowest MIC on *Candida albicans*. The *M. minutiflora* and *D. integrifolia* MIC was 3 mg/mL on *Escherichia coli* and *Shigella dysenteriae* A_1. *In vivo, D. integrifolia, D. preusii*, *T. okelensis* extract at 50 and 100 mg/kg bw and *M. minutiflora* 75 and 150 mg/kg bw significantly ($P< 0.01$) inhibited the intestinal charcoal transit. *D. integrifolia* 100 mg/kg bw exhibited the highest inhibition rate, 70%.

Conclusion: These results suggest that *D. integrifolia, D. preusii, M. minutiflora* and *T. okelensis* extracts possesses antimicrobial and antidiarrheal properties, could be effective for diarrhea treating, and could thus justify their use in traditional medicine to treat diarrhea. *D. integrifolia* could have the most efficiency antimicrobial properties.

Keywords*: Dichrocephala integrifolia, Dioscorea preusii, Melenis minutiflora, Tricalysia okelensis,* Antidiarrheal effects, Antimicrobial activity.

INTRODUCTION

Diarrhea is characterized as rapid movement of faecal matter through an intestine resulting in poor absorption of water, nutritive elements and electrolytes producing abnormal frequent evacuation of watery stools. Diarrhea is one of the main causes of the high mortality rate. According to the World Health Organization (WHO), 3 - 5 billion cases occur annually and approximately 5 million deaths are accountable to diarrhea. Over 2.5 million children under the age of five die annually from severe diarrheal diseases [1].

The disease may be caused by a wide array of agents such as entero-pathogenic microorganisms (*Shigella flexneri* and *Shigella dysenteriae, Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Candida albicans*), alcohol, irritable bowel syndrome, bile salts, hormones, secretory tumors and intoxication [2,3]. Plants have been a valuable source of natural products for maintaining human health for many years. The use of herbal drugs in the treatment of diarrhea is a common practice in many African countries. About 80 % of individuals from developed countries receive traditional medicines including compounds derived from medicinal plants.

Hence, medicinal plants can be exploited since it has been shown that they are important sources of new chemical substances with potential therapeutic effects [4].

However, there is limited scientific evidence supporting the potential use of these plants as antidiarrheal agents. The WHO suggested that medicinal plants would be the best source from which could be developed a variety of medications. We have therefore investigated the scientific basis for the efficacy of *Dichrocephala integrifolia, Dioscorea preusii, Melenis minutiflora,* and *Tricalysia okelensis* selected basing on their ethnomedicinal importance in diarrhea treatment.

MATERIALS AND METHODS

Test organisms

Three gram negative bacteria: *Escherichia coli* ATCC 35218, *Shigella dysenteriae* A1, *Salmonella typhi,* and the fungus *Candida albicans* were collected from Centre Pasteur of Cameroon. Their pure cultures were maintained in Muller-Hinton agar and stored at 4°C.

Animals

Young healthy Swiss albino mice (20-30 g) of either sex were bred in the University of Yaoundé I animal house. Animal housing and *in vivo* experiments was done according to the guidelines of the European Union on Animal Care (CEE Council 86/609) [5] that were adopted by the Institutional Committee of the Ministry of Scientific Research and Innovation of Cameroon. They were housed under laboratory natural temperature and day/light cycle. The animals were fed with standard diet and received water *ad libitum.*

Plant extracts

Fresh plants of *Dichrocephala integrifolia, Dioscorea preusii, Melenis minutiflora,* and *Tricalysia okelensis* were collected in June, 2011, from Batié subdivision, West Region of Cameroon. Botanic identification was performed at the Cameroon National Herbarium, Yaoundé, Cameroon, by comparison with the voucher kept under No. 5603/SRFcam, 16394/SRFcam, 34330/HNC, and 23930/SRFcam respectively.

The collected plants were shade dried and then grounded into powder. 600 g of the powder of each plant was macerated in water/ethanol (v/v) for 3 days. Then each macerate was filtered. The resulting filtrates were concentrated using a rotary evaporator and further dried in an oven at 40°C. The dried extracts obtained

were 27.80 g (4.63%), 21.30 g (3.55%), 26.20 g (4.36%) and 22.60 g (3.76%) respectively for *Dichrocephala integrifolia, Dioscorea preusii, Melenis minutiflora,* and *Tricalysia okelensis.*

Preliminary phytochemical analysis

The extract of each plant was subjected to qualitative chemical investigation for the identification of different phytochemical constituents [6].

Antimicrobial susceptibility

The susceptibility screenings of the extracts were done with the methods of dilution [7]. The sterilized medium (autoclaved at 121 °C for 15 min) maintained at 40°C was inoculated (1 mL/100 mL of medium) with the microorganism suspensions of 10^5CFU/mL (matched to McFarland barium sulphate standard) and poured into sterile petri dishes to give a depth of 3-4 mm.

Every discs (6 mm in diameter) were impregnated with 10 µL of each plant extracts dilution (0.39, 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50, 100 and 200 mg/mL) and placed on the surface of the inoculated Muller-Hinton agar. The inoculated plates were pre-incubated for 1 h at room temperature and then incubated at 37 °C for 18-24 h for antibacterial, and at 25°C for 48 h for antifungal activities. Ciprofloxacin (30 µg/disc) and Nystatin (100 µg/disc) were used as standard for antibacterial and antifungal activities respectively. Antimicrobial activity was evaluated by measuring the inhibition zone of the tested microorganism growth. All inhibition assays were made in triplicate.

Determination of minimal inhibitory concentration (MIC)

The determination of the minimal inhibitory concentration (MIC) of the hydro ethanolic plants extracts was carried out using the agar dilution method [8]. Two-fold dilutions of the extracts were prepared and 2 mL aliquot of different concentrations of the solution were added to 18 mL of pre-sterilized molten Muller-Hinton agar at 40 °C to give final concentration regimes of 50 to 0.05 mg/mL. The medium was then poured into sterile Petri dishes and allowed to solidify. The surfaces of the media were streaking with 18 h old microorganisms cultures. The plates were later incubated at 37 °C for 18-24 h for bacteria and at 25°C for 48 h fungi, after which they were examined for the microbial growth. The MICs were taken as the lowest concentration of extracts that prevent the visible growth of the tested microorganisms.

Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) assessment

To determine the minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) of the plant extracts against the microbes, the plates of the MIC that showed no growth of the microbes were sub-cultured by streaking using wire loop on sterile Muller Hinton agar plates. The plates were incubated at 37°C for 18-24 h and at 25°C for 48 h respectively for bacteria and fungi. The MBCs and MFC were taken as the lowest concentration of the extract that showed not microbial growth on the agar plates [9].

All the antimicrobial assays were performed under strict aseptic conditions to ensure consistency of all findings.

Antidiarrheal activity: castor oil-induced gastrointestinal motility

Ten groups of five mice each were fasted for 18 hours with free access to water. Each mouse orally received 0.5 mL of castor oil. One hour later, group I received 10 mL/kg bw distilled water; group III and group IV respectively received 50 and 100 mg/kg bw of *D. integrifolia* extract; group V and group VI respectively received 50 and 100 mg/kg bw of *D. preussii* extract; group VII and group VIII respectively received 50 and 100 mg/kg bw of *T. okelensis* extract; group IX and group X respectively received 75 and 150 mg/kg bw of *M. minutiflora* extract; After one hour all treated animals orally received 0.5 mL of charcoal meal (activated carbon 10 % in gum 5 %). 30 min after charcoal meal administration, the mice were sacrificed by cervical dislocation, and intestine was removed without stretching and placed length wise on moist filter paper and its total length measured. The distance moved by the charcoal from the pyloric sphincter to the caecum was measured. This distance was expressed as percentage of the small intestine total length [10].

Statistical analysis

The results were expressed as mean ± SEM. Statistical significance was determined by the one-way analysis of variance (ANOVA) followed by Dunnett's test using the software Graph Pad In Stat.

RESULTS

Preliminary phytochemical analysis

The preliminary phytochemical analysis of ethanol/water extracts of *D. integrifolia, D. preusii, M. minutiflora,* and *T. okelensis* revealed that these plants content saponins, phenolic compounds, coumarins, reduce sugar, polysaccharides and volatile oil, and no flavonoids. Tannins and alkaloids were present in *D. integrifolia, M. minutifllora,* and *T. okelensis* extract but not in *D. preussii* extract. *D. integrifolia* extract contents sterols which is absent in other extracts, whereas terpenoids are absent in *D. integrifolia* extract (Table 1).

Table 1: Preliminary phytochemical screening of ethanol/water extracts of *Dichrocephala integrifolia, Dioscorea preusii, Melenis minutifllora,* and *Tricalysia okelensis*

Secondary metabolites	*D. integrifolia*	*D. preussii*	*M. minutiflora*	*T. Okelensis*
Alkaloids	+	-	+	+
Anthraquinones	+	+	+	-
Coumarins	+	+	+	+
Flavonoids	-	-	-	-
Phenols	+	+	+	+
Polysaccharides	+	+	+	+
Reduce sugar	+	+	+	+
Saponins	+	+	+	+
Sterols	+	-	-	-
Tannins	+	-	+	+
Triterpens	-	+	+	+
Volatile oil	+	+	+	+

Key: +: present; -: absent.

Antimicrobial susceptibility

The results obtained from the disc diffusion assay showed that the plant extracts possess antimicrobial activities against the tested microorganisms (*S. typhi*, *S. dysenteriae*, *E. coli*, *C. albicans*) in dose-dependent manner. The highest inhibition activities were observed with the highest extract test dose 200 mg/mL (Table 2).

The inhibition diameter with *D. integrifolia* extract was 24 mm on both *S. typhi* and *C. albicans*, 19 and 17 mm respectively on *E. coli*, and *S. dysenteriae* A_1. *D. preussii* and *M. minutiflora* inhibition activities were respectively 22 and 19 mm against *S. typhi. D. preussii* and *M. minutiflora* extracts activities on *C. albicans* were 18 and 23 mm respectively. *T. okelensis* extract showed 20 mm inhibition activity only on *C. albicans.*

Minimal inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) obtained with *D. integrifolia* was 0.5, 1, 2 and 3 mg/mL respectively on *C. albicans, S. typhi*, *S. dysenteriae* and *E. coli*, 1 and 2 mg/mL with *D. preussii* respectively on *C. albicans* and *S. typhi,* 2 and 3 mg/mL with *M. minutiflora* respectively on *C. albicans* and *S. typhi* and 2 mg/mL with *T. okelensis* on *C. albicans* (Table 3).

Minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC)

The Minimal Bactericidal Concentration (MBC) values were ranges from 1-10 mg/mL. The lowest MFC value, 1 mg/mL was obtained on *C. albicans* with *D. integrifolia* extract. The highest MBC and MFC values, 10 mg/mL were recorded for *S. dysenteriae* and *C. albicans* with respectively *D. integrifolia* and *T. okelensis* (Table 3).

Intestinal transit time

The different extracts (50, 75, 100 and 150 mg/kg) significantly (p< 0.01) inhibited the intestinal transit of the charcoal meal in mice (Table 4). *D. integrifolia, D. preusii*, *T. okelensis* extracts at 50 and 100 mg/kg and *M. minutiflora* extract at 75 and 150 mg/kg significantly (p< 0.01) inhibited the charcoal intestinal transit.

The highest inhibition rate (70 %) was observed with 100 mg/kg of *D. integrifolia* extract and lowest inhibition rate (37.50 %) was obtained 100 mg/kg of *D. preusii* extract. The inhibition rate of the intestinal normal propulsion by the most tested extracts were comparable to (or more important than) the reference drug Loperamide.

Table 2: Antimicrobial susceptibilities (Inhibition diameter in mm) of *Dichrocephala integrifolia, Dioscorea preusii, Melenis minutiflora,* and *Tricalysia okelensis* extracts and standard drugs (Ciprofloxacin and Nystatin)

A	Microbial strains	Concentration of the extracts (mg/mL)										
Plant extracts		**0.19**	**0.30**	**0.78**	**1.56**	**3.12**	**6.25**	**12.50**	**25**	**50**	**100**	**200**
D. integrifolia	*S. typhi*	-	8.00	9.00	10.00	12.00	14.00	16.00	17.00	20.00	22.00	24.00
	E. coli	-	-	-	-	9.00	10.00	11.00	12.00	13.50	15.00	17.00
	S. dysenteriae	-	-	-	-	9.00	10.00	12.00	14.00	16.00	17.00	19.00
	C. albicans	-	8.00	10.50	11.00	12.00	13.00	14.50	16.00	18.00	19.50	24.00
M. minutiflora	*S. typhi*	-	-	-	9.00	10.00	12.00	14.00	15.00	17.00	19.00	19.00
	E. coli	-	-	-	-	-	-	-	-	-	-	-
	S. dysenteriae	-	-	-	-	-	-	-	-	-	-	-
	C. albicans	-	-	-	9.00	10.50	12.00	15.00	17.00	18.00	20.00	23.00
D. preussii	*S. typhi*	-	-	-	9.00	10.00	11.00	13.00	14.00	16.00	19.00	22.00
	E. coli	-	-	-	-	-	-	-	-	-	-	-
	S. dysenteriae	-	-	-	-	-	-	-	-	-	-	-
	C. albicans	-	-	-	8.00	9.00	10.00	12.00	13.50	14.50	16.00	18.00
T. okelensis	*S. typhi*	-	-	-	-	-	-	-	-	-	-	-
	E. coli	-	-	-	-	-	-	-	-	-	-	-
	S. dysenteriae	-	-	-	-	-	-	-	-	-	-	-
	C. albicans	-	-	8.00	10.00	11.00	12.50	14.00	15.50	16.50	17.50	20.00
B												
Standard drugs												
Ciprofloxacin	*S. typhi*		36									
30 µg/mL	*E. coli*		38									
	S. dysenteriae		35									
Nystatin	*C. albicans*		32									
100 µg/mL												

E. coli: Escherichia coli S. typhi: Salmonella typhi, S. dysenteriae: Shigella dysenteriae A_1 *C. albicans: Candida albicans*

Table 3: Minimal Inhibitory (MIC) and bactericidal (MBC) or fungicidal (MFC) concentrations (mg/mL) of the ethanol/water extract of *Dichrocephala integrifolia, Dioscorea preusii, Melenis minutiflora,* and *Tricalysia okelensis* on *Salmonella typhi, Shigella dysenteriae* A_1, *Escherichia coli* and *Candida albicans*

Plant extract	*Salmonella typhi*			*Escherichia coli*			*Shigella dysenteriae* A_1			*Candida albicans*		
	MIC	**MBC**	**MBC/MIC**	**MIC**	**MBC**	**MBC/MIC**	**MIC**	**MBC**	**MBC/MIC**	**MIC**	**MFC**	**MFC/MIC**
D. integrifolia	1.00	2.00	2.00	3.00	4.00	1.30	2.00	10.00	5.00	0.50	1.00	2.00
D. preussii	2.00	5.00	2.50	-	-	-	-	-	-	1.00	4.00	4.00
M. munitiflora	3.00	5.00	1.70	-	-	-	-	-	-	2.00	4.00	2.00
T. okelensis	-	-	-	-	-	-	-	-	-	2.00	10.00	5.00

Table 4: Effect of ethanol/water extract of *Dichrocephala integrifolia, Dioscorea preusii, Melenis minutifllora,* and *Tricalysia okelensis* on castor oil-induced intestinal transit

Traitements	LSI (cm)	LCM (cm)	PI	% inhibition
Distilled water	48.90 ±2.56	43.80 ±2.47	89.75±3.80	-
*Di*50	50.00 ±1.58	26.70 ±2.39**	53.40±3.54 **	40.50
*Di*100	48.08 ± 2.22	13.20 ± 2.39**	27.45±3.73 **	70.00
*Dp*50	48.30 ±2.44	19.00 ± 3.53**	39.33±8.22 **	55.00
*Dp*100	53.46 ±1.95	30.00 ±1.81**	56.11±2.43 **	37.50
*Mm*75	47.20 ± 1.60	19.60 ± 1.93**	41.52±4.30 **	54.00
*Mm*150	45.68 ± 1.77	22.28 ± 1.47**	48.77±2.30 **	46.00
*To*50	45.10 ± 1.10	22.80 ±1.67 **	50.55±4.54 **	43.40
*To*100	43.40 ± 1.74	18.90±1.12**	43.56±2.18 **	51.50
Lop5	30.00± 1.15	13.70± 0.58**	45.67± 0.78**	43.73

LSI = Length of Small Intestine; LCM = Length of Charcoal Meal; PI = Peristaltic Index; Values are means ± SEM (n=5);

significant difference: **P<0.01 compared to control. *Dichrocephala integrifolia* extract 50 mg/kg bw (*Di*50) and 100 mg/kg bw (*Di*100); *Dioscorea preusii* extract 50 mg/kg bw (*Dp*50) and 100 mg/kg bw (*Dp*100); *Melenis minutiflora extract 75 mg/kg bw (Mm75)* and 100 mg/kg bw (*Mm*150); *Tricalysia okelensis* extract 50 mg/kg bw (*To*50) and 100 mg/kg bw (*To*100); Loperamide 5 mg/kg (Lop5).

DISCUSSION

Dichrocephala integrifolia, Dioscorea preusii, Melenis minutiflora, and *Tricalysia okelensis* are some medicinal plants used in folk medicine in West Cameroon to treat diarrhea. In order to verify their antidiarrheal activities, the *in vitro* antimicrobial effect on *Salmonella typhi*, *Escherichia coli*, *Shigella dysenteriae* A_1 and *Candida albicans*, and the *in vivo* activities on castor oil-induced small intestinal motility were studied.

The ethanol-water extract of *D. integrifolia, D. preusii, M. minutiflora,* and *T. okelensis* showed dose-dependent activity against all the tested microbial strains with inhibition activity varied from one plant to another. *D. integrifolia* extract was the most active drug against all the tested microbial strains. *D. integrifolia, D. preusii, M. minutiflora,* and *T. okelensis* extract showed significant activity against *C. albicans*; whereas *D. preusii,* and *M. minutiflora* extracts showed significant antibacterial activity against *Salmonella typhi* but were found to be ineffective against *Shigella dysenteriae* type 1 and *Escherichia coli*. *T. okelensis* extract was inactive on *Salmonella typhi*, *Shigella dysenteriae* A_1, and *Escherichia coli*.

Various phytochemicals compounds like alkaloids, saponins, tannins, reducing sugar, phenols, polysaccharides, anthraquinons, coumarins, triterpens and sterols were present in *D. integrifolia, D. preusii, M. minutiflora,* and *T. okelensis*. The antimicrobial susceptibility of these medicinal plants could be attributed to some phytochemicals compounds which possess antimicrobial activities, such as anthraquinones [11], tannins [12], polysaccharides [13], alkaloids [14], saponins, phenols [15], and sterols [16]. The antibacterial activity of the plant extracts can be attributed to single bioactive compound or combined action of many compounds contained in the extracts [17].

Castor oil induced diarrhea in mice probably through its most active metabolite, ricinoleic acid by irritating the gastrointestinal tract mucosa and reducing sodium ion and chloride ion permeability, resulting in increased intestinal motility followed by diarrhea [18]. It also activates adenylate cyclase or mucosal cAMP mediated active secretion, endogenous prostaglandin E and F and nitric oxide formation [19,20].

In this study, the loperamide was adopted as the standard drug, which is at present one of the most efficient and widely employed antidiarrheal drugs [21]. The loperamide slows down transit in the intestine, reduces colon flow rate, and possesses antimotility and antisecretory properties [22]. The ethanol/water extracts of *D. integrifolia, D. preusii, M. minutiflora,* and *T. okelensis* inhibited gastrointestinal propulsion in the castor oil-induced transit. The antidiarrheal properties of the ethanol/water extracts may be due to the presence of secondary metabolites such as tannins which could denature proteins in the intestinal mucosa by forming protein tannates complex. The protein tannates make the intestinal mucosa more resistance and hence, reduce the peristaltic movements and intestinal secretion [18].

CONCLUSION

In conclusion, the results of this investigation revealed that ethanol-water extracts of *D. integrifolia, D. preusii, M. minutiflora,* and *T. okelensis* were effective antimicrobial agents against certain microorganisms implicated in either typhoid fever and/or other gastrointestinal infectious diseases such as diarrhea and dysentery. *D. integrifolia* extract has shown the best bactericidal, fungicidal and antidiarrheal activity. The results provide the rationale for the use of these extracts by traditional healers as antidiarrheal drug

REFERENCES

1. Semwal BC, Goyal A, Varshney V. Antidiarrheal activity of *Rhododendron arborium* leaves in wistar rats. Int J Pharm Res Bio-Sci 2014;3(3):591-600.
2. Brijesh S, Tetali P, Birdi TJ. Study of effect of anti-diarrheal medicinal plants on enteropathogenic *Escherichia coli* induced interleukin-8 secretion by intestinal epithelial cells. Altern Med Stud 2011;(1)e16:64-9.
3. Yakubu MT, Opakunle FK, Salimon SS, Ajiboye TO, Bamisaye FA, Quadri AL. Antidiarrheal activity of aqueous leaf extract of *Ceratotheca sesamoides* in rats. Bangladesh J Pharmacol 2012;7:14-20.
4. Akuodor GC, Muazzam I, Usman-Idris M, Megwas UA, Akpan JL. Evaluation of the antidiarrheal activity of methanol leaf extract of *Bombax Buonopozense* in rats. Ibnosina J Med Biomed Sci 2011;3(1):15-20.
5. Smith JA, van den Broek FAR, Martorel JCL, Hackbarth H, Ruksenas O, Zeller W. FELASA working grp and "principles and practice in ethical review of animal experiments across Europe: summary of the report of a FELASA Working Group on ethical evaluation of animal experiments." Lab Anim 2007;41(2):143-60.
6. Trease GE, Evans WC: Pharmacognosy, 12th ed. London: Baillere Tindall; 1983.
7. Sheela XQR, Ramani VA. Antimicrobial screening of *polygonum barbatum* leaf extract. J Curr Pharm Res 2011;3(3):117-8.
8. Akinyele TA, Okoh OO, Akinpelu DA, Okoh AI. *In-Vitro* antibacterial properties of crude aqueous and n-hexane extracts of the husk of *Cocos nucifera*. Mol 2011;16(3):2135-45.
9. Spencer ALR, Spencer JFT. Public Health Microbiology: Methods and protocols. New Jersey; Human Press Inc; 2004. p. 325-7.
10. Lakshminarayana M, Shivkumar H, Rimaben P, Bhargava VK. Antidiarrhoeal activity of leaf extract of *Moringa Oleifera* in experimentally induced diarrhoea in rats. Int J Phytomed 2011;3(2011):68-74.
11. Irimpan MT, Jolly CI, Sheela D. A Study of the phytochemical composition and antibacterial activity of *Holostemma adakodien* schultes. Int J Pharm Tech Res 2011;2(3):1208-10.
12. Lacmata ST, Kuete V, Dzoyem JP, Tankeo SB, Ngo Teke G, Kuiate JR, *et al*. Antibacterial activities of selected Cameroonian plants and their synergistic effects with antibiotics against bacteria expressing MDR phenotypes. Evidence-Based Complementary Altern Med 2012;1-11.
13. Murugesan S, Pannerselvam A, Tangavelou AC. Phytochemical screening and antimicrobial activity of the leaves of *Memecylon umbellatum* burm. F J Appl Pharm Sci 2011;01(01):42-5.
14. Rahman MA, Ahsna T, Islam S. Antibacterial and antifungal properties of methanol extract from the stem of *Argyreia argentea*. Bangaladesh J Pharmacol 2010;5:41-4.
15. Šiljegović JD, Stojković DS, Nikolić MM, Glamočlija JM, Soković MD. Antimicrobial activity of aqueous extract of *Laetiporus sulphureus* (bull.: fr.) murill. Nat Sci 2011;120:297-303.
16. Sridhar S, Deepa T, Elamathi R, Kavitha R, Kamalakannan, Suresh Kumar J. Antimicrobial activities of leaf extracts of *Sapindus emarginatus* Vahl. Int J Pharm Tech Res 2012;4(1):392-7.
17. Sunayana V, Vadivukkarasi A, Rajendran T, Francis X, Natarajan E. Antibacterial potential of *Plectranthus amboinicus* (Lour) Spreng. A study *in vitro*. J Swamy Bot Club 2003;20:55-8.
18. Haque A, Abdullah CS, Romana B, Rafique B, Zia-ul-Huda, Hossain SF, et al. Evaluation of anti-diarrheal and anti-diabetic activities of the stem, barks and leaves of the plant Vernonia cinerea (Family: Asteraceae). J Appl Pharm Sci 2013;3(01):069-072.
19. Capasso F, Mascolo N, Izzo AA, Gaginella TS. Dissociation of castor oil-induced diarrhea and intestinal mucosal injury in rat: effect of NGnitro-L-arginine methyl ester. Br J Pharmacol 1994;113(4):1127-30.
20. Anup M, Saikat D, Subhash CM. *In vivo* evaluation of antidiarrhoeal activity of the seed of *Swietenia macrophylla* King (Meliaceae). Trop J Pharm Res 2007;6(2):711-6.
21. Balogun SO, Tanayen JK, Ajayi AM, Ibrahim A, Ezeonwumelu JOC, Oyewale AA, *et al*. Preliminary evaluation of anti-diarrheal, ulcer-protective and acute toxicity of aqueous ethanolic stem bark extract of *Ficus trichopoda* in experimental rodents. Asian J Med Sci 2011;3(1):37-42.
22. Theoderau V, Floramont J, Hachet T, Bueno L. Absorptive and motor components of anti-diarrhoeal action of loperamide: an *in vivo* study in pigs. Gut 1991;32:1355-9.