



**Journal of
Medicinal Plant Research**

Volume 10 Number 43, 17 November, 2016

ISSN 1996-0875



*Academic
Journals*

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ARTICLES

- Pharmacological activities of methanol extract of *Phyllanthus acidus* pulp** 790
Farjana Afrin, Sujan Banik and Mohammad Salim Hossain
- In vitro antibacterial activity of *Cymbopogon citratus*, *Eucalyptus citriodora*, *Lippia multiflora*, *Melaleuca quinquenervia* essential oils and Neco® on extended-spectrum β -lactamases producing or non-producing bacterial strains** 796
Jean-Fabrice Yala, Rolande Mabika Mabika, Brahima Camara, Alain Souza, Alexis Nicaise Lepengue, Tuo Seydou, Daouda Koné and Bertrand M'batchi
- Acute toxicity study of *Sphaeranthus bullatus* used as herbal tea in Tanzania** 805
Okumu Ochanga and Musa Chacha

Full Length Research Paper

Pharmacological activities of methanol extract of *Phyllanthus acidus* pulp

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Received 8 April, 2015; Accepted 13 November, 2015

The methanolic extract of *Phyllanthus acidus* pulp was screened in the present study to explore pharmacological activities on laboratory animals. The hypoglycemic activity was assessed by glucose tolerance test and the antidiarrheal activity was evaluated by castor oil induced diarrhea inhibition method. The analgesic and anesthetic activity was determined by Tail immersion method and Thiopental sodium induced sleeping time method, respectively. The extract showed significant ($P < 0.05$) blood glucose activity at 500 mg/kg of body weight. The extract at both doses showed pronounced antidiarrheal activity and inhibited the average number of defecation by 54.24% ($P < 0.05$) and 10.17% at both doses, respectively. In Tail-immersion test, the extract showed significant ($P < 0.05$) analgesic activity at both doses. The extract also showed good CNS anesthetic activity to the experimental animal. It was concluded that from this study, the methanolic extract of *P. acidus* have potential different pharmacological activities and justify its use in folkloric medicines.

Key words: *Phyllanthus acidus*, hypoglycemic, anti-diarrheal, analgesic, anesthetic.

INTRODUCTION

Plants have been used for mankind as remedies from the very beginning of civilization. To date, they play an important role in health care for about 80% of the world's population. They have long been a very important source of new drugs. Many plant species have been screened for substances with therapeutic activity. Bangladesh possesses a rich flora of medicinal plant. Out of the estimated 5000 species of phanerogams and pteridophytes growing in this country, more than a thousand are regarded as having medicinal properties (Mia and Rahman, 1990). A large number of plants included in Euphorbiaceae family have been investigated all over the world.

Euphorbiaceae plants, which are investigated in Bangladesh show wide range of secondary metabolites including antibacterial, hypoglycemic, antidiarrheal, anaesthetic and analgesic compounds.

Phyllanthus acidus, a plant belongs to the family Euphorbiaceae. It is commonly known as the Otaheite gooseberry, Malay gooseberry, Tahitian gooseberry, Country gooseberry, Star gooseberry, Starberry, West India gooseberry, Grosella (in Puerto Rico), Jimbilin (in Jamaica), damsel (in Grenada), karamay (in the Northern Philippines), or simply gooseberry tree. It is mostly cultivated for ornamentation and locally name as Arboroi (Bengali). This tropical or subtropical species is found

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throughout Asia and also has a home in the Caribbean region, Central and South America (Janick and Robert, 2008).

The plant is an intermediary between shrubs and tree with edible small yellow berries fruits. There are many important chemical constituents found in this plant. For example, fruits contain ascorbic and tartaric acids and tannin, root bark contains tannin (18%), gallic acid, saponin, lupeol and a crystalline substance (Chopra et al., 1992), stem bark includes a phytosterol, different from lupeol (Ghani, 2003). The plant is reported to have many uses. Latex is credited with emetic and purgative activity, bark is used to treat bronchial catarrh and is a popular local treatment. Roots are used to alleviate asthma and also used to treat psoriasis of the feet, leaf decoction is applied to treat urticarial. On the other hand, fruit is used as a laxative and the antioxidants present in fruit have a hepatoprotective effect on liver (Reddy, 2014; Jain et al., 2011). Extract has shown nematocidal activity against the pine wood nematode and have possibility to provide treatment of cystic fibrosis of the lungs (Sousa et al., 2007). Leaves are useful to treat blood vomiting, piles, small pox, fever, itching and gum infection (Subhadrabandhu, 2001). Furthermore, it is known to possess anticancer (Mahidol et al., 2002), antibacterial (Melendez and Capriles, 2006) and antiviral (Direkbusarakom et al., 1996) activities. The fruit is extensively used in Ayurvedic system as a liver tonic and blood purifier. It is also used in several vitiated conditions of jaundice, piles, constipation, vomiting, bronchitis, biliousness and urinary concretions (Kirtikar and Basu, 1987).

All these previous studies revealed that there are a lot of phytochemical constituents which may possess some effect on antidiarrheal, anesthetic or analgesic like action. Previous *in vitro* studies reveal the possibilities of such kind of *in vitro* model studies. However, the present study intended to investigate the pharmacological like hypoglycemic, antidiarrheal, analgesic and anesthetic activity of methanolic extract of the pulp of *P. acidus* in swiss-albino mice, which is abundantly growing in Bangladesh.

MATERIALS AND METHODS

Chemicals and drugs

Glibenclamide (Dibenol), Loperamide hydrochloride (Imotil) are brand products of Square Pharmaceuticals Ltd, Morphine Sulphate (Morphinex[®]), Diazepam (Easium) and Thiopental sodium (Anestho) product of Popular Pharmaceuticals, Oponin Pharma and Incepta Pharmaceuticals Ltd, respectively were purchased from the local market of Bangladesh. All other chemicals and reagents used throughout the whole study were of analytical grade.

Collection of plant

The fresh fruits of *P. acidus* were collected from comilla,

Bangladesh in August 2012. It was identified and authenticated by National Herbarium of Bangladesh, Mirpur, Dhaka (voucher specimens of the collection DACB Accession no. 38200) and have been deposited in Bangladesh National Herbarium (BNH) for future references.

Preparation of methanol extracts

The collected plant parts (fruits-pulp) were separated from seed. They were air-dried for one week. Then, the plant parts were pulverized into a coarse powder with the help of a suitable grinder. About 110 g of powdered material was taken in a clean glass container and soaked in 300 ml of 80% methanol (Merck, Germany) and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture then filtrated by a piece of clean and white cotton material. Then, it was filtered using Whatman No. 42 filter paper. After obtaining clear filtrates, they were then evaporated by using traditional spontaneous natural vaporization method at room temperature. It rendered an oily concentrate of yellowish black color. The crude extract was stored at 4°C until analysis.

Experimental animals

Swiss-albino mice of either sex, aged 4 to 5 weeks, obtained from the Animal Resource Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B) were used for the experiments. They were housed in standard polypropylene cages and kept under controlled at room temperature (24 ± 2°C; relative humidity 60 to 70%) in a 12 h light-dark cycle and fed ICDDR, B formulated rodent food and water (*ad libitum*). They were kept before the test for at least 3 to 4 days in the environment where the experiment took place, due to their sensitivity with the environmental changes.

Hypoglycemic activity

A Glucose Tolerance Test (GTT) is the most commonly used method to evaluate the hypoglycemic activity in which glucose is given in the systemic circulation. Blood sample were taken afterward to determine how quickly it is cleared from the blood. In the present study, hypoglycemic effect of methanolic extract of *P. acidus* at 250, 500, and 1000 mg/kg doses were examined and compared relative to that of control and standard group. Glibenclamide was used as a standard drug in this experiment (Pari and Venkateswaran, 2002). Ten experimental animals were randomly selected and divided into five groups consisting of 2 mice in each group. Each group received an individual treatment. In order to administer the extract at doses of 250, 500, and 1000 mg/kg body weight of mice, the exactly weighed extracts were measured, respectively and triturated in unidirectional way by adding small amount of Tween-80 (a suspending agent). After proper mixing of extract and suspending agent, normal saline was slowly added with it. The ultimate or final volume of the suspension was made up to 3 ml. To stabilize the suspension, it was stirred well by using vortex mixture. For the preparation of standard (Glibenclamide) at the dose of 10-mg/kg body weight, 10 mg tablet was dissolved into 3 ml normal saline (0.9% NaCl) (Kasiviswanath et al., 2005). At zero hour test samples, control (1% Tween-80 solution in saline) and Glibenclamide (in standard groups) were administered orally by means of a long needle with a ball shaped end. After 60 min, all groups were treated with 10% glucose solution (2 g/kg body weight). Then, after 30, 60 and 120 min of glucose loading, blood samples were collected from tail vein. By using glucometer blood glucose level was measured (Kamaeswara

Table 1. Hypoglycemic activity of *P. acidus* pulp in experimental animals.

Test group	Dose (mg/kg)	Plasma glucose level (mmol)			
		0 h	0.5 h	1 h	2 h
Control	1% tween 80	6.2±0.23	10.5±0.42	7.3±0.23	5.7±0.23
Standard	10	5.45±0.33	4.8±0.21*	3.5±0.23*	1.7±0.23*
<i>P. acidus</i>	250	4.05±0.31	5.0±0.26	5.55±0.23	4.7±0.23
<i>P. acidus</i>	500	3.6±0.22	4.8±0.28*	4.6±0.33*	4.0±0.27*
<i>P. acidus</i>	1000	5.85±0.23	4.45±0.20	4.15±0.23	5.55±0.23

Values are expressed as mean ± SEM. *Significant as compared to standard (Glibenclamide) and control p<0.05.

et al., 2001).

Antidiarrheal activity

The anti-diarrheal activity of the methanolic extract of *P. acidus* was evaluated using the method of castor oil induced diarrhea in mice (Shoba and Thomas, 2001). According to this method, each mouse was fed with 1 ml of highly pure analytical grade of castor oil which would induce diarrhea. The numbers of fecal stools were recorded for each individual mouse. The observations of the experimental groups were compared against that of the control to evaluate the anti-diarrheal activity of the samples. The experimental animals were divided into control, positive and test groups, with two mice in each group. The control group received vehicle (1% tween in normal saline) at dose 10 ml/kg body weight orally. Loperamide was given to the positive control group at the dose of 50 mg/kg orally. The test group received methanolic extract of *P. acidus* at the doses of 200 and 400 mg/kg body weight. Each animal was placed in an individual cage; the floor lining was changed at every hour. Diarrhea was induced by oral administration of castor oil to each mouse after the above treatment. During an observation period of 5 h; the number of diarrhoeic feces excreted by the animals was recorded (Maikere et al., 1989).

Analgesic activity

Evaluation of central analgesic activity was carried by tail immersion method using morphine as a positive control. The changes in sensitivity of test animal due to analgesic activity of drugs are measured in this method. A constant heat stress is applied to rat tail, which acts as pain stimulus. When the stimulus exceeds the threshold, rat shows a quick withdrawal of its tail. Time taken by the rat to withdraw the tail is termed as tail immersion time (Upudha et al., 2007). Analgesic compound elongates this responding time. The test rats were orally fed with test materials whereas the positive control received morphine subcutaneously. From 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55°C. The reaction time is the time required by the mice to deflect their tails. The time required to withdraw the tail was recorded. The experimental animals were divided into control, positive and test groups, with two mice in each group. Control and test samples (at doses of 200 and 400 mg/kg body weight of mice) were given orally. At zero hour, 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55°C. The reaction time is the time required by the mice to deflect their tails. The first reading is rejected and the reaction time is recorded as a mean of the next three reading. A latency period of 20 s was defined as complete analgesia. The latent period of tail-flick response was determined before and 0, 30, 60 and 90 min after the administration of drugs. A

30 min interval was given to ensure proper absorption of the administered substances. Then morphine solution (a dose of 2 mg/kg for 25 mg mice) was administered subcutaneously to the mice. After 30, 60 and 90 min, the tail immersion time was measured.

Anesthetic activity

The method described by Williamson et al. (1996) was followed for this study. The test animals were divided in four groups consisting of two mice each group. Group I was the control group, Group II was the standard group, whereas group III (A) and III (B) were experimental groups. The experimental groups were treated with test samples prepared with normal saline water and tween-80 at doses of 200 and 400 mg/kg body weight, standard group was administered intraperitoneally with Diazepam (1 mg/kg) containing normal saline water, while the control group was administered normal saline water containing 1% Tween 80 solution. 30 min later thiopental sodium (40 mg/kg body weight) was administered intraperitoneally to all the groups to induce sleep. The onset of sleep and total sleeping time were recorded.

Statistical analysis

Descriptive statistics were calculated for all variables by using SPSS software package (version 19.0). All of the values were expressed as mean ± standard error of mean (SEM). Data analysis among the groups was compared using one-way ANOVA analysis. P value of <0.05 was considered as significant.

RESULTS

Hypoglycemic activity

The result of hypoglycemic screening of pulp extract of *P. acidus* shows effective blood glucose lowering activity at dose of 250, 500, and 1000 mg/kg, but more significant at 500 mg/kg dose in comparison with standard (Glibenclamide) and control group in statistical analysis greater than dose effect in Table 1.

Antidiarrheal activity

The methanolic extract of pulp of *P. acidus* was subjected

Table 2. Effect of methanol extract of *P. acidus* pulp and Loperamide on castor oil induced diarrhea in mice.

Test group	Dose (mg/kg)	No. of diarrheal faeces in 5 h	% Inhibition
Control	10 ml/kg	29.5	
Loperamide	50	9.0	69.49*
<i>P. acidus</i>	200	13.5	54.24*
<i>P. acidus</i>	400	26.5	10.17

*Significant as compared to control $p < 0.05$.

Table 3. Analgesic activity of *P. acidus* pulp by tail immersion method.

Test group	Dose (mg/kg)	Reaction time (min)			
		0	30 m	60	90
Control	Vehicle	3.45 ± 0.33	4.35 ± 0.23*	2.41 ± 0.19*	4.23 ± 0.31*
Morphine	2	5.14 ± 0.33	19.17 ± 0.43*	20.0 ± 0.21*	16.0 ± 0.21*
<i>P. acidus</i>	200	3.42 ± 0.33	3.30 ± 0.13*	13.9 ± 0.31*	6.62 ± 0.32*
<i>P. acidus</i>	400	4.13 ± 0.33	11.11 ± 0.25*	5.90 ± 0.11*	15.50 ± 0.44*

Values are expressed as mean ± SEM. *All data were found to be significant at 5% level of significance where $p < 0.05$

Table 4. Effect of methanol extract of *P. acidus* pulp on thiopental sodium-induced sleep.

Test group	Treatment	Dose (mg/kg)	Time of onset of sleep (min)	Total sleeping time (min)
I (Control)	1% tween 80	Vehicle	16	107.8
II (Standard)	Diazepam	1	9	158
III	Test drug A	200	27	155
IV	Test drug B	400	20	139

to castor oil induced diarrhea experiment at doses 200 and 400 mg/kg and compared with relative to that of control and standard (loperamide) group. The obtained data showed that the pulp extract exhibited significant ($p < 0.05$) anti-diarrheal activity at dose 200 mg/kg compared to control group (Table 2).

Analgesic activity

In the tail immersion method of analgesic study, the methanolic extract showed significant analgesic activity at both doses of 200 and 400 mg/kg (Table 3) when compared with relative to that of control and standard (morphine) group.

Anesthetic activity

Table 4 presented the values of anesthetic activity of the methanolic extract of pulp of *P. acidus* was tested at 200 and 400 mg/kg doses and compared with relative to that of control and standard (Diazepam) group.

DISCUSSION

Diabetes, this chronic metabolic disorder, is a major public concern in the whole world characterized by high blood glucose concentration due to insulin deficiency or frequently combined with insulin resistance. In recent years, its increasing rate is very high in developing countries like Bangladesh. Traditionally, many plants are used for treatment and management of diabetes mellitus throughout the world (Marles and Farnsworth, 1995). In the present study, we explore the hypoglycemic activity of methanolic extract of *P. acidus* pulp at different doses level, 250, 500 and 1000 mg/kg and the finding of the study showed the effective hypoglycemic activity which is more significant (4.8 ± 0.28 , 4.6 ± 0.33 and 4.0 ± 0.27) at 500 mg/kg dose in comparison with standard (Glibenclamide). Obviously, there is less dose effect on hypoglycemic activities which may be overdose saturation of receptor activities. But according to the statistical data, dose at 500 mg/kg is significant against the control values. Recinolic acid, an active metabolite of castor oil, which causes diarrhea due to increase in peristaltic movement of the small intestine leading to alteration in electrolyte

permeability in the intestinal mucosa and it is a hypersecretory response. Since the methanolic extract of pulp of *P. acidus* fruitfully inhibited castor oil induced diarrhea and assume that this action was mediated by an antisecretory mechanism (Stewart et al., 1975).

The crude methanolic extract of *P. acidus* pulp exhibited significant ($p < 0.05$) anti-diarrheal activity at dose 200 mg/kg (54.24%) compared to control group. Here also the low dose effect, which reveals that, this crude extract has more effect at lower dose instead of high dose. In this experiment, the crude methanolic extract of *P. acidus* pulp showed significant analgesia (3.30 ± 0.13 , 13.9 ± 0.31 , and 6.62 ± 0.32) at different time interval (30, 60 and 90 min) in the dose of 200 mg/kg body weight compared to standard (19.17 ± 0.43 , 20.0 ± 0.21 , and 16.0 ± 0.21) at the same time intervals. A relevant phenomenon involved for the effect of analgesic against thermal noxious stimuli may be produced through opioid receptors or through modulation of several neurotransmitters. The activities of analgesic and anti-diarrheal have been attributed due to their phytochemical compounds of saponin, terphenoids, flavonoids and phenolic contents. The phytochemical screening of this plant by other researchers (Sukanya et al., 2013; Manjulatha et al., 2014) revealed that *P. acidus* possessed the maximum phytochemicals like alkaloids, flavonoids, saponins, tannins and phenolic compounds. CNS anesthetic activity was performed for the pulp of *P. acidus* at 200 and 400 mg/kg doses and was administered to the test animal (swiss-albino) for thiopental sodium induced sleeping time test.

The pulp extract slightly increase the thiopental sodium induced sleeping time shown in Table 4. The time of onset of sleep was 16 min in control group, whereas in experimental group, it was 27 and 20 min at doses of 200 and 400 mg/kg body weight, respectively. The total sleeping time was about 155 and 139 min at the doses of 200 and 400 mg/kg body weight, respectively, while it was 107.8 min in control group. So, it is clear from the observation that the methanolic extract at 200 mg/kg body weight showed better CNS anesthetic activity than 400 mg/kg body weight dose. All the experimental values are effective at lower dose and it may be due to the effectiveness of the active phytoconstituents at low dose and further investigations are required to establish the plant's activates over a wide range.

Conclusion

The results of the present study led us to the inference that the pulp extract possess significant hypoglycemic, anti-diarrheal, analgesic, and anesthetic properties. Since this plant is used in traditional medicine, the extracts should be further explored for its phytochemical profile to identify active constituent responsible for hypoglycemic, anti-diarrheal, analgesic, and anesthetic activities,

especially at the lower dose.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors thank the Department of Pharmacy, Noakhali Science and Technology University for providing necessary logistic support to carry out the research. They would also like to thank Md. Hasan Kawsar, Associate Professor, Department of Pharmacy, State University of Bangladesh for his support to conduct different pharmacological tests.

REFERENCES

- Chopra RN, Nayer SL, Chopra IC (1992). Glossary of Indian Medicinal Plants. Council Sci. Ind. Res. 3:7-246.
- Direkbusarakom S, Herunsalee A, Yoshimizu M, Ezura Y (1996). Antiviral activity of several Thai traditional herb extracts against fish pathogenic viruses. Fish Path. 31:209-213.
- Ghani A (2003). Medicinal Plants of Bangladesh-Chemical constituents and uses. Asiatic Soc. Bangladesh 2:502-504.
- Jain NK, Lodhi S, Jain A, Nahata A, Singhai AK (2011). Effects of *Phyllanthus acidus* (L.) Skeels fruit on carbon tetrachloride-induced acute oxidative damage in livers of rats and mice. J. Chinese Integr. Med. 9(1):49-56.
- Janick J, Robert EP (2008). The Encyclopedia of Fruit and Nuts. CABI: 373. Available at: https://books.google.com.ng/books?id=cjHCoMQNkcgC&pg=PA373&redir_esc=y&hl=en#v=onepage&q&f=false
- Kamaeswara RB, Giri R, Kesavulu MM, Apparao C (2001). Effect of oral administration of bark of *Pterocarpus santalinus* L. on blood glucose level in experimental animals. J. Ethnopharmacol. 74:69-74.
- Kasiviswanath R, Ramesh A, Kumar KE (2005). Hypoglycemic and antihyperglycemic effect of *Gmelina asiatica* Linn. in normal and in alloxan induced diabetic rats. Biol. Pharm. Bull. 28:729-732.
- Kirtikar KR, Basu BD (1987). Indian Medicinal Plants. Lalit. Mohan. Basu. 2(3):2227-2228.
- Mahidol C, Prawat H, Prachywarakorn V, Ruchirawat S (2002). Investigation of some bioactive Thai medicinal plants. Phytochem. Rev. 1:287-297.
- Maikere FR, Van PL, Mutwewingabo A, Habiaremye FX (1989). Study of Rwandese medicinal plants used in the treatment of diarrhea. J. Ethnopharmacol. 26:101-9.
- Manjulatha K, Manjari M, Sarita k, Nasir I, Oruganti HS (2014). Phytochemical and antioxidant studies on fruits of *phyllanthus* species. Vri Phytomed. 2(3):81-85.
- Marles JR, Farnsworth NR (1995). Antidiabetic plants and their active constituents. Phytomedicine 2(2):123-89.
- Melendez PA, Capriles VA (2006). Antibacterial properties of tropical plants from Puerto Rico. Phytomedicine 13:272-276.
- Mia MMK, Rahman M (1990). Preliminary ethnomedicinal studies at Sandwip island of Bangladesh. Tradit. Med. 104-108.
- Pari L, Venkateswaran S (2002). Hypoglycaemic activity of *Scopariadulcis* L. extract in alloxan induced hyperglycemic rats. Phytother. Res. 16(7):662-664.
- Reddy O (2014). *Phyllanthus acidus*-Indian gosseberry.
- Shoba FG, Thomas M (2001). Study of antidiarrhoeal activity of four medicinal plants in castor oil induced diarrhea. J. Ethnopharmacol. 76:73-76.
- Sousa M, Ousingsawat J, Seitz R, Puntheeranurak S, Regalado A,

- Schmidt A (2007). An Extract from the Medicinal Plant *Phyllanthus acidus* and its isolated compounds induced Airway Chloride Secretion: A potential treatment for Cystic Fibrosis. *Mol. Pharmacol.* 71:366-376.
- Stewart JJ, Gagarella TS, Bass P (1975). Actions of ricinolic acid and structurally related fatty acids of the gastrointestinal tract, effects on smooth muscle contractility in vitro. *J. Pharmacol. Exp. Ther.* 195:347-54.
- Subhadrabandhu S (2001). Under-utilized tropical fruit of Thailand. RAP Publication, Thailand. 37.
- Sukanya MK, Shimi S, Aruna SR (2013). Phytochemical analysis, antimicrobial screening and antihelminthic properties of *phyllanthus emblica*. *Int. J. Pharm. Biol. Sci.* 4(4):55-64.
- Upudha AL, Rathnakar UP, Udupa S (2007). Anti-inflammatory, Anti-pyretic and Analgesic effect of *Tamrindus indica*. *Indian Drug* 44(6):466-470.
- Williamson E, Okpako DT, Evans FJ (1996). Selection, preparation and pharmacological evaluation of plant material (Vol. 1). John Wiley & Sons.

Full Length Research Paper

In vitro antibacterial activity of *Cymbopogon citratus*, *Eucalyptus citriodora*, *Lippia multiflora*, *Melaleuca quinquenervia* essential oils and Neco® on extended-spectrum β -lactamases producing or non-producing bacterial strains

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Received 12 October, 2016; Accepted 11 November, 2016

This study aims at assessing the *in vitro* activity of *Cymbopogon citratus*, *Eucalyptus citriodora*, *Lippia multiflora*, *Melaleuca quinquenervia* essential oils and the biobactericide Neco® on extended-spectrum β -lactamases producing or non-producing bacterial strains (ESBL) isolated at the Armed forces hospital of Libreville. The aromatoqram and antibiogram were respectively assessed by the agar well diffusion method and agar disc method. Thus, the minimum inhibitory concentration and minimum bactericidal concentration were determined by the microdilution method in liquid medium.

The aromatoqram revealed that the biobactericide Neco® induced the largest inhibition diameters (28.42 - 43.27 mm) in all strains, followed by *E. citriodora* (26.96 - 36.12 mm) and *L. multiflora* (15.32 - 41.42 mm) essential oils. In contrast, *M. quinquenervia* (9.83 - 26.64 mm) and *C. citratus* (6.82 - 14.97 mm) essential oils had the smallest inhibition diameters. Furthermore, the comparison of aromatoqram and antibiogram activities generally revealed that activities are better with essential oils than with antibiotics. In addition, no significant differences were observed between ESBL producing or non-producing strains. At the end this study, the antibacterial activity of *C. citratus*, *E. citriodora*, *L. multiflora*, *M. quinquenervia* essential oils and the biobactericide Neco® were highlighted. However, the efficiency of these activities is dependent on the intrinsic composition of the plant.

Key words: Essential oils, extended spectrum β -lactamase (ESBL), multi-resistance, biobactericide, antibacterial activity.

INTRODUCTION

The third millennium is marked by the emergence of cases of bacterial resistance to antibiotics. Indeed, many

studies have showed the presence of resistant bacteria (RB), highly resistant bacteria (HRB) and multi-

bacteria (RB), highly resistant bacteria (HRB) and multi-resistant bacteria (MRB) (Fournier et al., 2012; Jing-yi Zhao et al., 2014; Li et al., 2015) to antibiotics, in hospitals as well as in communities. These resistances have a direct impact on the effectiveness of the antibiotherapy by making it difficult. Thus, β -lactamase producing bacteria, by their multi-resistance to antibiotics pose a serious public health problem.

Building on this alarming fact, it seems wise to focus research on the discovery of new molecules. These should be different from conventional antibiotics and have different mechanisms of action (Falconner et al., 2009). Clearly, in recent years, natural resources and more particularly aromatic plants have been the subject of renewed interest in the search for antimicrobial molecules. The choice was directed to essential oils, natural complex mixtures of volatile secondary metabolites, extracted from aromatic plants (Kalemba and Kunicka, 2003) which present numerous bioactive properties. Indeed, essential oils of *Chrysocoma ciliata* L. (Afolayan and Ashafa, 2009), *Cymbopogon proximus* and *Ocimum canum* (Bassolet et al., 2001), of *Prangos asperula* Boissier (Hilan et al., 2009) *Inula viscosa*, *Salvia officinalis* and *Laurus nobilis* (Kheyar et al., 2014) and *Lavandula officinalis* showed antimicrobial activities. Some showed antifungal activity (Kalemba and Kunicka, 2003; Koné et al., 2010; Doumbouya et al., 2012; Kassi et al., 2014; Kouamé et al., 2015). Thus, they are used in food preservation (Mahanta et al., 2007; Kouamé et al., 2015).

Essential oils of *Cymbopogon citratus*, *Eucalyptus citriodora*, *Lippia multiflora*, *Melaleuca quinquenervia* and the biobactericide Neco® showed antifungal activity on truck farm mushrooms and banana (*Pythium* sp. and *Fusarium oxysporum*, *Mycosphaerella fijiensis*, *Deightoniella torulosa* etc) in Côte d'Ivoire (Camara et al., 2010; Koné et al., 2010; Doumbouya et al., 2012).

In this study, we propose to assess *in vitro* the antibacterial activity of *C. citratus*, *E. citriodora*, *Lippia multiflora*, *Melaleuca quinquenervia* essential oils and the biobactericide Neco® on beta-lactamase producing or non-producing Gram- bacteria. This work falls within the scope of promotion of aromatic molecules for the purpose of using them in medicine.

MATERIALS AND METHODS

Essential oils (EH)

In this work, we were provided with five (5) essential oils (EH) by the Laboratory of Plant Physiology, Faculty of Biosciences, University Félix HOUPOUËT-BOIGNY of Cocody-Abidjan (Côte d'Ivoire). The extraction of these species was performed according

to the method described by several authors in previous studies (Camara et al., 2010; Koné et al., 2010; Doumbouya et al., 2012). Their different characteristics are noted in Table 1. Among these oils, the biobactericide Neco®, is a product used as a trademark and sold by the University Félix HOUPOUËT-BOIGNY (Kassi et al., 2014).

Bacterial strains

For this study, eight bacterial strains cryopreserved in the laboratory of Molecular and Cellular Biology (LABMC) were tested (Table 2). Their selection criteria were based on the fact that these bacteria are frequently isolated in hospital environment, and are responsible for various pathologies. Thus, these strains are carrier or non-carrier of antibiotic resistance genes (ATB).

Bacterial cultures or overnight cultures

From cryopreserved stocks, 50 μ l were taken and transferred into tubes containing brain-heart infusion broth (BHI) (Bioméieux, France). Then, the tubes were incubated at 37°C for 18 to 24 h.

Assessment of the antibacterial activity of essential oils by the diffusion method

The aromatogram method was used to determine the inhibitory activity of EH. From overnight cultures, successive decimal dilutions were performed at (10^{-1} to 10^{-3}), in order to obtain standardized inocula (approximately 10^5 to 10^6 cells/ml). These were seeded by flooding on Mueller-Hinton agar (MH). Then the plates were dried in the laminar flow hood. Afterwards, wells were made in the agar using Pasteur pipettes and 50 μ l of the different pure extracts were deposited. Finally, the dishes were incubated at 37°C for 18 to 24 h. After incubation, the inhibition diameters were measured.

Assessment of antibiotic sensitivity: Comparative test

The determination of resistance phenotypes based on the sensitive, intermediate and resistant trilogy of bacterial strains was made by the diffusion method on MH agar medium. For this purpose, an antibiogram was conducted with antibiotic discs (ATB) belonging to five different families (Table 3).

Determination of the minimum inhibitory concentration (MIC)

The microdilution method in liquid medium in 96-well microplates was used to assess bacteria growth inhibition parameters by essential oils (EH). Indeed, two-fold geometric dilutions were performed (from 1/2 to 1/256). In order to obtain a homogeneous solution, the EHs were diluted in a Tween 20 solution added with the culture medium. The microplate was covered with parafilm and incubated at 37°C for 18 to 24 h. The lowest concentration of essential oils inhibiting any growth visible to the naked eye after the incubation period was the MIC. Thus, three trials were conducted for each of the strains and the MIC value was the average of both tests.

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Table 1. Features of the essential oils studied.

Scientific names	Families	Abbreviation
<i>Cymbopogon citratus</i>	Poaceae	Cymbo
<i>Eucalyptus citriodora</i>	Myrtaceae	Euca
<i>Lippia multiflora</i>	Verbenaceae	Lippia
<i>Melaleuca quinquenervia</i> L.	Myrtaceae	Melaq
Neco®	/	Neco®

Table 2. Bacterial strains tested.

Family	ESBL-producing	ESBL-non-producing
Enterobacteriaceae	<i>Escherichia coli</i>	<i>Escherichia coli</i>
	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>
	<i>Enterobacter aerogenes</i>	<i>Enterobacter cloacae</i>
Acinetobacteriaceae /Pseudomonaceae	<i>Acinetobacter baumannii</i>	<i>Pseudomonas aeruginosa</i>

Table 3. Names of the different antibiotic discs.

Antibiotic families	Names of molecules used	Disc initials
Beta-lactams: 3 rd generation cephalosporin	Cefotaxime	CTX
Bêta-lactamines: 4 th generation cephalosporin	Cefepime	FEP
Phenicols	Chloramphenicol	C
Tetracyclines	Doxycycline	DO
Aminosides	Gentamicin	GMI
Quinolones	Ofloxacin	OFX

Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration or MBC was determined by seeding on Lauria-Bertani (LB) medium by spreading 100 µl samples of wells not showing growth in the microplate. The lowest essential oil concentration having decimated 99.99% of the starting population, after 24 h of incubation at 37°C corresponded to the MBC. The intrinsic activity of the different EHs was determined based on the ratio MBC/MIC named α (alpha). In fact, if $1 \leq \alpha \leq 2$, the effect is bactericidal and if $4 \leq \alpha \leq 16$, the effect is bacteriostatic (Kpodekon et al., 2013; El amri et al., 2014). Moreover, for any value of α superior to 16, the activity is said to be tolerant.

Statistical analysis

The single-factor analysis of variance was used for comparing the inhibitory capacity of essential oils. Duncan's multiple range test was used to compare in pairs the different essential oils. All these were done with the XLSTAT 2014 software and Excel 2013.

RESULTS

Assessment of essential oil (EH) activities

In this study, the antimicrobial activity was assessed by

observing the inhibiting capacity of the five (5) essential oils at the same concentration on the bacteria. The results obtained are shown in Table 4. The values recorded are the averages of the three tests.

Indeed, all the essential oils showed a significant inhibitory effect against the studied microorganisms. However, a variation in efficiency in terms of inhibition zones depending on the type of germ and oil concerned was observed.

Activities of oils on *E. coli* strains

The results obtained in this study showed that the 5 tested oils had an inhibitory activity on the ESBL producing or non-producing *E. coli* strains. Indeed, inhibition diameters ranging from 6.96 ± 1.67 to 41.42 ± 8.12 mm and 6.82 ± 1.42 to 28.42 ± 7.08 mm respectively were observed for *E. coli* ESBL+ and *E. coli* BLSE-. Also, the single-factor analysis of variance showed that there was a significant difference ($p < 0.0001$ and $p = 0.0002$) between the different oils used against *E. coli* ESBL+ and BLSE- bacteria. As for Duncan's test, it showed that there was no significant difference between the biobactericide

Neco® and *E. citriodora* and *L. multiflora* oils. However, a difference was found between *C. citratus* and *M. quinquenervia* essential oils and the other above-mentioned oils. Similarly, the parity check showed no significant difference between the biobactericide Neco® and *E. citriodora* oil, between *L. multiflora* and *M. quinquenervia* oils and between those of *M. quinquenervia* and *C. citratus* for *E. coli* BLSE-. Overall, no significant difference ($p = 0.33 > 0.05$) existed between the behavior of ESBL-producing and non-producing strains.

Activities of oils on *Klebsiella*

Considering the results, an inhibitory effect was recorded for all oils regarding the two tested *Klebsiella*. Obviously, inhibition zones ranging between 7.54 ± 1.87 and 29.34 ± 5.22 mm were observed for *K. pneumoniae* carrying resistance genes with diameters ranging from 8.96 ± 2.69 to 31.40 ± 5.61 mm for the other *Klebsiella*. Moreover, for the 5 oils, significant differences ($p \geq 0.002$) were obtained for both strains. Thus, Duncan's test showed a significant difference against *K. pneumoniae* ESBL+ respectively between the biobactericide Neco® and *E. citriodora* oil, *C. citratus*, *M. quinquenervia* and *L. multiflora* oils. Moreover, the same result was obtained between species *L. multiflora* and *C. citratus*. Moreover, for *K. pneumoniae* BLSE-, the paired comparison between oils, showed a significant difference between the biobactericide Neco® and *C. citratus*, *M. quinquenervia* and *L. multiflora* oil. Also, the same observation was made between *E. citriodora*, *L. multiflora* and *M. quinquenervia* species. No significant differences ($p > 0.05$) existed between the behavior of ESBL+ strains and the other BLSE-.

Activity of oils on bacteria of the genus *Enterobacter*

The average inhibition diameters of species obtained regarding bacteria of the genus *Enterobacter* ranged from 7.86 ± 1.86 to 43.27 ± 9.32 mm.

Concerning the species *Ent. cloacae* BLSE-, the value of diameters ranged between 7.86 ± 1.86 - 31.00 ± 12.94 mm. From a statistical point of view, there was a significant difference ($p = 0.011 < 0.05$) between the 5 species against this bacterium. The Duncan's test conducted showed that there was a significant difference for *Ent. cloacae* between Neco® volatile and *E. citriodora*, and *C. citratus* and *M. quinquenervia* oils respectively on the one hand. On the other hand, between *L. multiflora* and *C. citratus*.

For the species *Ent. aerogenes*, which is ESBL+, diameters in the range of 8.12 ± 2.56 to 43.27 ± 9.32 mm were observed. Also, the statistical analysis revealed a significant difference ($p < 0.0001$). Duncan's multiple range test performed, showed no significant difference for species *L. multiflora*, *M. quinquenervia* and *C. citratus*.

And also, for *M. quinquenervia* and *C. citratus* oils against *Ent. aerogenes*. In general, no significant difference ($p = 0.33$) existed between the behavior of ESBL-producing bacteria and the other bacteria.

Activity of oils on *P. aeruginosa* BLSE-

The results revealed that all the species tested showed an activity against *P. aeruginosa* (Table 4). Thus, the diameters observed ranged between 14.97 ± 13.29 and 36.12 ± 10.82 mm. Furthermore, the statistical analysis showed that there was no significant difference ($p = 0.109$) between these oils for this strain.

Activity of oils on *A. baumannii* ESBL+

The five species also showed an effective activity on *A. baumannii*. Diameters varying between 11.50 ± 0.57 and 42.62 ± 11.89 mm were recorded. The best diameter was obtained with the biobactericide Neco® followed by the *E. citriodora*, *M. quinquenervia*, *L. multiflora* and *C. citratus* species which showed a significant difference ($p = 0.005$). The parity test showed that there was no significant difference between the biobactericide and *E. citriodora*, *M. quinquenervia* and *L. multiflora* oils. The same result was also observed between *M. quinquenervia* and *C. citratus*.

Comparison between aromatogram and antibiogram

The sensitivity of bacteria against six (6) antibiotics (ATB) was assessed by the standard disc diffusion method. The measures of inhibition halos obtained are summarized in Table 5.

Considering the results, it appears that both *E. coli* strains showed a resistance profile to the different antibiotics tested, except for gentamicin for *E. coli* BLSE-. The *Klebsiella* strains, ESBL+ strains as for it, were resistant to chloramphenicol and cefepime while for BLSE- it was resistant to doxycycline only. In contrast, *Ent. aerogenes* was resistant to cephalosporins, to doxycycline and ofloxacin. Similarly, *Ent. cloacae* was resistant to chloramphenicol only. Concerning the species *A. baumannii*, resistance to two ATB (cefepime and chloramphenicol) was recorded. Furthermore, a resistance phenotype was observed for *P. aeruginosa* against ATBs of the cephalosporin family, doxycycline, chloramphenicol and ofloxacin.

By comparing the activity of essential oils and that of ATBs, the oils showed better inhibition diameters. Indeed, the results recorded revealed that the diameters of antibiogram ranged from 0.00 ± 0.00 and 31.68 ± 0.21 mm (Table 5), while those of aromatogram ranged from 6.82 ± 1.42 to 43.27 ± 9.32 mm for all strains put together (Table 4).

Table 4. Antibacterial activity of different essential oil against bacterial strains.

Strains	Inhibition diameters of essential oils (mm)					
	Essential oils					
	Neco	Euca	Melaq	Cymbo	Lippia	
ESBL+	<i>E. coli</i>	38.25± 6.09 ^a	31.92± 0.51 ^a	17.30± 6.71 ^b	6.96± 1.67 ^c	41.42± 8.12 ^a
	<i>K. pneumoniae</i>	29.34± 5.22 ^a	28.89± 1.93 ^{a,b}	14.75± 5.93 ^b	7.54± 1.87 ^c	15.58± 3.32 ^{b,c}
	<i>A. baumannii</i>	42.62± 11.89 ^a	32.85± 2.75 ^{a,b}	26.64± 8.95 ^b	11.50± 0.57 ^b	21.57± 6.67 ^{b,c}
	<i>Ent. aerogenes</i>	43.27± 9.32 ^a	27.29± 4.07 ^b	10.07± 2.27 ^c	8.12± 2.56 ^c	16.45± 4.68 ^c
ESBL -	<i>E. coli</i>	28.42± 7.08 ^a	27.29± 4.13 ^a	9.83± 2.11 ^{b,c}	6.82± 1.42 ^c	15.32± 4.08 ^b
	<i>K. pneumoniae</i>	34.37± 6.58 ^a	36.12± 10.82 ^a	15.76± 10.12 ^a	14.97± 13.29 ^a	23.43± 12.69 ^a
	<i>Ent. cloacae</i>	31.00± 12.94 ^a	28.88± 1.83 ^a	11.05± 2.74 ^{b,c}	7.86± 1.86 ^c	22.48± 9.76 ^{a,b}
	<i>P. aeruginosa</i>	31.40± 5.61 ^a	26.96± 0.93 ^{a,b}	12.83± 2.84 ^{b,c}	8.96± 2.69 ^c	18.64± 9.61 ^{a,b,c}

*All the values shown are the averages of 3 trials (n=3) avec ± standard deviation. *On the same line the values having the same letter are not significantly different from a<b<c.

Table 5. The antibiogram.

Strains	Inhibition diameters (mm)						
	CTX (30 µg)	FEP (30 µg)	C (30 µg)	DO (30 U.I)	GMI (15 µg)	OFX (5 µg)	
ESBL+	<i>E. coli</i>	7.79±0.71 ^R	12.39±0.71 ^R	14.13±0.08 ^R	11.55±2.29 ^R	12.9±5.95 ^R	17.23±1.92 ^I
	<i>K. pneumoniae</i>	31.66±0.71 ^S	9.57±0.62 ^R	21.25±0.77 ^I	0.00±0.00 ^R	18.52±1.99 ^S	29.53±1.44 ^S
	<i>A. baumannii</i>	18.94±0.09 ^I	13.61±0.56 ^R	0.00±0.00 ^R	23.54±4.18 ^S	20.68±1.77 ^S	27.97±1.46 ^S
	<i>Ent. aerogenes</i>	9.83±0.25 ^R	14.12±0.71 ^R	25.79±0.04 ^S	11.57±0.64 ^R	16.53±0.46 ^S	0.00±0.00 ^R
ESBL -	<i>E. coli</i>	12.27±0.86 ^R	18.13±1.00 ^I	14.00±0.64 ^R	8.80±3.96 ^R	18.63±0.35 ^S	0.00±0.00 ^R
	<i>K. pneumoniae</i>	28.30±3.42 ^S	29.45±2.20 ^S	26.93±1.35 ^S	10.03±1.80 ^R	17.70±0.32 ^S	23.79±2.00 ^S
	<i>Ent. cloacae</i>	27.76±0.71 ^S	31.13±0.71 ^S	18.05±0.57 ^R	18.04±1.97 ^I	17.32±2.00 ^S	31.68±0.21 ^S
	<i>P. aeruginosa</i>	9.93±0.74 ^R	15.05±0.40 ^I	10.40±0.88 ^R	12.90±1.24 ^R	21.96±2.33 ^S	10.70±1.84 ^R

R, Resistant bacterium; I, intermediate bacterium; S, sensitive bacterium.

Determination of MICs

The different minimum inhibitory concentrations (MICs) obtained for each of the oils tested are shown in Table 6. Each of these oils showed different activities depending on their nature, but also on the bacterial strains tested. Thus, the biobactericide Neco® and the natural species *E. citriodora* and *L. multiflora* showed strong inhibitory capacities except for *K. pneumoniae* and *P. aeruginosa* respectively for Neco® and *L. multiflora*. Furthermore, these three (3) oils, gave interesting MICs ranging between 3 ± 1.41 and $96 \pm 45.25 \mu\text{l.ml}^{-1}$. In contrast, *C. citratus* and *M. quinquenervia* species showed very high MICs or even an absence for some strains (Table 6). And therefore, they had low and very low inhibitory capacities (Table 6).

Determination of MCB and assessment of the MBC/MIC

In general, the biobactericide Neco® and the *E. citriodora* species had a bacteriostatic activity against the strains

tested (Table 8). The values of minimum bactericidal concentrations (MBCs) were included in a range of 16 ± 0.00 to $96 \pm 45.25 \mu\text{l/ml}$ for the first oil. Similarly, the MBC values were in the range 12 ± 5.66 to $192 \pm 90.51 \mu\text{l/ml}$, respectively (Table 7).

Considering the results, the *M. quinquenervia* species therefore had a bactericidal intrinsic activity overall. However, the values of its concentrations were very high and were between 64 ± 0.00 and $\geq 256 \pm 0.00 \mu\text{l/ml}$. However, the EH of *L. multiflora* also had a bactericidal activity against most strains studied. With the difference that the assessment of MBCs showed values ranging from 32 ± 0.00 to $192 \pm 90.51 \mu\text{l/ml}$, which are close to the MIC values obtained for this essential oil (Table 6). Furthermore, the only oil of which no effect was observed during this study was *C. citratus*. Thus, no MBC was recorded for all tested bacteria.

DISCUSSION

Through this study, the antibacterial activities of five (5) essential oils (HE) were assessed. In the light of the

Table 6. Different values of essential oils minimum inhibitory concentration (MICs).

Strains	MIC ($\mu\text{l.ml}^{-1}$)					
	Essential oils					
	Neco	Euca	Melaq	Cymbo	Lippia	
ESBL+	<i>E. coli</i>	6 ± 2.83	4±0.00	96±45.25	>256	12±5.66
	<i>K. pneumoniae</i>	64±0.00	6±2.83	160±135.76	>256	6±2.83
	<i>A. baumannii</i>	4±0.00	6±2.83	128±0.00	256±1.41	12± 22.63
	<i>Ent. aerogenes</i>	3±1.41	6±2.83	96±45.25	>256	48± 8.49
ESBL -	<i>E. coli</i>	6±2.83	6±2.83	32±11.31	>256	4±0.00
	<i>K. pneumoniae</i>	4±0.00	16±0.00	192±90.51	>256	12±5.66
	<i>Ent. cloacae</i>	10±8.49	10±8.49	256±0.00	128±0.00	8±0.00
	<i>P. aeruginosa</i>	16±0.00	24±11.31	96±45.25	256±0.00	96±45.25

Interpretation of MICs: MIC < 48 $\mu\text{l.ml}^{-1}$: Strong inhibitory capacity; 48 $\mu\text{l.ml}^{-1}$ < CMI < 96 $\mu\text{l.ml}^{-1}$: Average inhibitory capacity; 96 $\mu\text{l.ml}^{-1}$ < CMI < 256 $\mu\text{l.ml}^{-1}$: Low inhibitory capacity; CMI \geq 256 $\mu\text{l.ml}^{-1}$: Very low or nil inhibitory capacity.

Table 7. Essential oils minimum bactericidal concentrations.

Strains	Concentration ($\mu\text{l.ml}^{-1}$)					
	Essential oils					
	Neco	Euca	Melaq	Cymbo	Lippia	
ESBL+	<i>E. coli</i>	32±0.00	12±5.66	192±90.51	NT	32±0.00
	<i>K. pneumoniae</i>	64±0.00	48±22.63	/	NT	24±11.31
	<i>A. baumannii</i>	32±0.00	16±0.00	256±0.00	/	64±0.00
	<i>Ent. aerogenes</i>	16±0.00	64±0.00	192±90.51	NT	32±0.00
ESBL -	<i>E. coli</i>	96±45.25	64±0.00	64±0.00	NT	96±45.25
	<i>K. pneumoniae</i>	16±0.00	64±0.00	256±0.00	NT	48±22.63
	<i>Ent. cloacae</i>	64±0.00	192±90.51	/	/	192±90.51
	<i>P. aeruginosa</i>	24±11.31	64±0.00	128±0.00	/	128±0.00

NT: None tested; /: no MBC.

Table 8. Nature of the intrinsic activity of the essential oils studied.

Strains	Nature of the effect					
	Essential oils					
	Neco	Euca	Melaq	Cymbo	Lippia	
ESBL+	<i>E. coli</i>	Bacteriostatic	Bactericidal	Bactericidal	ND	Bactericidal
	<i>K. pneumoniae</i>	Bactericidal	Bacteriostatic	Nd	ND	Bactericidal
	<i>A. baumannii</i>	Bacteriostatic	Bacteriostatic	Tolerant	ND	Bactericidal
	<i>Ent. aerogenes</i>	Bacteriostatic	Bactericidal	Bactericidal	ND	Bactericidal
ESBL -	<i>E. coli</i>	Bacteriostatic	Bacteriostatic	Bactericidal	ND	Tolerant
	<i>K. pneumoniae</i>	Bacteriostatic	Bacteriostatic	Bacteriostatic	ND	Bacteriostatic
	<i>Ent. cloacae</i>	Bacteriostatic	Tolerant	ND	ND	Tolerant
	<i>P. aeruginosa</i>	Bactericidal	Bactericidal	Bactericidal	ND	Bactericidal

results, it appears that the inhibitory effect of these species, against the studied bacteria, is heterogeneous. Thus, the biobactericide Neco® and the *Eucalyptus citriodora* (*E. citriodora*) and *Lippia multiflora* (*L.*

multiflora) oils present the highest efficiencies while *Melaleuca quinquenervia* (*M. quinquenervia*) and *Cymbopogon citratus* (*C. citratus*) oils have lower efficiencies as compared to the first three ones.

The identification of the antibacterial activities of these natural substances corroborates the antimicrobial capacity of the essential oils described by many works (Benkherara et al., 2011; Chahboun et al., 2015; Yang et al., 2015). In fact, the recorded biological activities can be explained first by the chemical composition of these oils which is very complex, but also, by the quantitative and qualitative variability of its components (Gabriel et al., 2013; El amri et al., 2014). Indeed, according to the works of El Amri et al. (2014), due to the variability of quantities and profiles of essential oil components, it is likely that their antimicrobial activity is not attributable to a single mechanism, but to several sites of action at the cellular level. For these authors, the mode of action of essential oils depends primarily on the type and characteristics of active components, particularly their hydrophobic property that enables them to penetrate the phospholipid bilayer of the bacterial cell membrane. This would therefore induce a conformational change of the membrane, a chemo-osmotic disturbance and ion (K^+) leakage.

The antibacterial property of *E. citriodora* oil was reported by Traoré et al. (2013). This study showed that this EH has an activity against *E. coli* and *S. aureus* and that this activity might come from its high content of aldehyde (76.33%), called citronellal. Moreover, these same authors suggest that improved activity may be observed by testing the pure oil, as confirmed by the results of this study. Indeed, diameters of 10 mm were obtained with dilute species of *E. citriodora* for *E. coli* by Traoré et al. (2013). In contrast, for pure species, diameters of 15.32 ± 4.08 and 41.42 ± 8.12 mm were recorded for both *E. coli* phenotypes tested in our study.

Moreover, the antimicrobial activity of *L. multiflora* might be due to the presence of molecules of sesquiterpene (β -caryophyllene), geraniol, γ -terpinene, para-cymene, thymol, carvacrol, and 1.8-cineole (Soro et al., 2015).

For the biobactericide Neco®, which active matter was obtained from *O. gratissimum* (*O. gratissimum*) fresh leaves and used as pesticide (Kassi et al., 2014). The results of the works of Nakamura et al. (1999) and Kpodekon et al. (2013) have shown that the antibacterial activity might be correlated to its high concentration in phenolic compounds particularly thymol.

As for the species *C. citratus*, its antibacterial virtue might be linked to the presence of major components such as the neral/geraniol frequently called citral (Koba et al., 2004; Mahanta et al., 2007; Koba et al., 2009).

However, the analysis of the results clearly highlights a significant difference in activity of the five oils on the bacterial strains tested. These differences can be explained by the intrinsic properties of their constituent molecules. Therefore, the antimicrobial activity would depend on the lipophilicity of the carbon chains and functional groups concerned (phenol>aldehydes>ketones>alcohols>ethers>

hydrocarbons) (Folashade and Omoregie, 2012; Gabriel et al., 2013). The fact that the volatile extracts of *L. multiflora* and Neco® are mainly composed of terpene and phenol, and that the one of *E. citriodora*, of aldehyde molecules, might likely explain the large inhibition diameters observed and therefore the best activities obtained during this study. Also, the fact that all strains are sensitive to it might be explained by the wide spectrum of antibacterial activity of these molecules (Kheyar et al., 2014).

However, for the *C. citratus* oil, despite the major presence of an aldehyde, low activities were obtained. Thus, the pure extract tested showed activities ranging from 6.82 ± 1.42 to 14.97 ± 13.29 mm. Similar results were obtained for dilute species of *C. proximus* and *canum* (Bassole et al., 2001). These results suggest that the intrinsic characteristics of the studied strains (Bourkhiss et al., 2007; El Amri et al., 2014), or the volatile extract and the existence of the synergy/antagonist effect between the different majority and minority components (Yang et al., 2015), might modulate the efficiency of the volatile extract. In addition, the variability factor of the chemical composition that could be related to ecology, to the harvest period in the year and especially to soil structure (Kpodekon et al., 2013), might justify the results observed.

Finally, the antifungal efficiency of the species of *M. quinquenervia*, was demonstrated by the works of Camara et al. (2010), and Doumbouya et al. (2012). Indeed, these authors showed that the fungicidal effect of this EH could be attributed to terpene molecules which are its most constituents and whose main monoterpenes were 1.8-cineole (46.5%), α -pinene (11.9%) and viridiflorol. However, in this study antibacterial activities were revealed. In analogy to the composition of *L. multiflora* oil, this activity observed could be related to the presence of 1.8-cineole, component common to both species, as well as by the presence of α -pinene. Indeed, Ngom et al. (2014) explained that these hydrocarbons such as α -pinene, sabinene are known for their antimicrobial capacity.

Among the eight bacteria tested, four are extended-spectrum β -lactamase producing (ESBLs). Considering the results of antibiogram, most of ESBL+ strains are not only resistant to cephalosporins, but also to other families of antibiotics tested. The same observation is made for the other strains of the study. Comparing the results of aromatoigram and antibiogram, it is clear that the essential oils tested show the best activities. According to the literature, similar results were obtained by Bassole et al. (2001) for *L. multiflora* by comparing its activity with that of gentamicin and penicillin. These authors explained that at a smaller concentration by 2.5 times, the EH induced better activities. Similarly, Kheyar et al. (2014) have obtained the same results with *Inula viscosa* L., *Salvia officinalis* L. species. Indeed, diameters varying from 15.5 to 31.5 mm and 10.12 to 24 mm respectively

for *Inula viscosa* L., *Salvia officinalis* L., oils have been recorded; they were higher than those of the different antibiotics tested (6 to 31 mm).

However, Tibyangye et al. (2015) have determined the inferior inhibition diameters for the species of *Ocimum suave* compared to reference antibiotics ciprofloxacin and nitrofurantoin, tested on uropathogens. In fact, the average activities of this oil ranged from 16 to 22 mm. In contrast, they ranged from 13 to 29 mm and 11 to 26 mm respectively for ciprofloxacin and nitrofurantoin.

These results can be understood by the mechanism of action of essential oils, as the inhibitory activity of volatile extracts might be correlated with the existence of several modes of action related to their active components described by many authors (Kouamé et al., 2015). Indeed, the antibacterial activity appears to be influenced by combined action at many levels on the bacterial structure (Bouhdid et al., 2012). But also by the synergistic effect between the different components in spite of the presence of the majority molecules (Ngom et al., 2014). That certainly might explain the lack of difference in behavior, at the significance level between ESBL+ and ESBL-.

In the light of the results, the species of *L. multiflora* and *M. quinquenervia* are bactericidal while Neco® and *E. citriodora* are bacteriostatic. However, only *C. citratus* has no *in vitro* intrinsic activity. The bactericidal and bacteriostatic properties demonstrated during this study are in accordance with those of (Kpodekon et al., 2013) in which the *O. gratissimum* oil is bactericidal and those of Haddouchi et al. (2009) with the *Thymus fontanesii* species. Overall, the effect of essential oils is bacteriostatic. However, most of their chemical elements might have bactericidal properties (Benkherara et al., 2011).

Conclusion

Finally, this study has highlighted the *in vitro* antibacterial activity of essential oils of *C. citratus*, *E. citriodora*, *L. multiflora*, *M. quinquenervia*, and Neco® on medically relevant bacteria. Based on these results, these natural species have antimicrobial activities that depend on the intrinsic qualitative and quantitative composition of each species.

Their antibacterial activities on all strains and especially on those referred to as resistant bacteria suggests bits of solutions to deal with the thorny issue of emergence of resistant and multi-resistant strains. These species seem likely to be potential candidates for anti-infective therapies. However, *in vivo* studies are needed in order to assess all their antibacterial capacities.

Conflicts of Interests

The authors have not declared any conflict of interests.

REFERENCES

- Afolayan AJ, Ashafa AO (2009). Chemical composition and antimicrobial activity of the essential oil from *Chrysocoma ciliata* L. leaves. *J. Med. Plants Res.* 3:390-394.
- Bassole I, Ouattara A, Nebie R, Ouattara CAT, Kabore Z, Traore SA (2001). Composition chimique et activités antibactériennes des huiles essentielles des feuilles et des fleurs de *Cymbopogon proximus* (STAPP) et d'*Ocimum canum* (SIMS). *Pharm. Méd. Tradit. Afr.* 11:37-51.
- Benkherara S, Bordjiba O, Djahara AB (2011). Etude de l'activité antibactérienne des huiles essentielles de la Sauge officinale: *Salvia officinalis* L. sur quelques entérobactéries pathogènes. *Rev. Synthèse* 23:72-80.
- Bouhdid S, Abrini J, Baudoux D, Manresa A, Zhiri A (2012). Les huiles essentielles de l'origan compact et de la cannelle de Ceylan : pouvoir antibactérien et mécanisme d'action. *J. Pharm. Clin. Sci.* 31(3):141-148.
- Bourkhiss B, Ouhssine M, Hnach M, Bourkhiss M, Satrani B, Farah A (2007). Composition chimique et bioactivité de l'huile essentielle des rameaux de *Tretraclinis articulata*. *Bull. Soc. Pharm. Bordeaux* 146:75-84.
- Camara B, Dick E, Sako A, Kone D, Boye MAD, Aké S, Ano A (2010). Lutte biologique contre *Deightoniella torulosa* (Syd.) Ellis, par application des huiles essentielles d'*Eucalyptus platyphylla* F. Muell. et de *Melaleuca quinquenervia* L. *Phytothérapie* 8(4):240-244.
- Chahboun N, Esmail A, Abed H, Barrahi M, Amiyare R, Berrabeh Oudda M, Ouhssine M (2015). Evaluation de l'activité bactériostatique d'huile essentielle de la *Lavandula officinalis* vis-à-vis des souches d'origine clinique résistantes aux antibiotiques. *J. Materials Environ. Sci.* 6(4):1186-1191.
- Doumbouya M, Abo K, Lepengue A, Camara B, Kanko K, Aidara D, Kone D (2012). Activité comparées *in vitro* de deux fongicides de synthèse et de deux huiles essentielles, champignons telluriques des cultures maraichères en Côte d'Ivoire. *J. Appl. Biosci.* 50:3520-3532.
- El Amri J, Elbadaoui K, Zai T, Hayate B, Saïd C, Taj Imolk A (2014). Etude l'activité antibactérienne des huiles essentielles de *Teucrium capitatum* L et de l'extrait de *Silène vulgaris* sur les différentes souches testées. *J. Appl. Biosci.* 82:7481-7492.
- Falconer SB, Brown ED (2009). New screens and targets in antibacterial drug discovery. *Curr. Opin. Microbiol.* 12:497-504.
- Folashade KO, Omoregie EH (2012). Essential oil of *Lippia multiflora* Moldenke: *J. Appl. Pharm. Sci.* 2(1):15-23.
- Fournier S, Brun-Buisson C, Jarlier V (2012). Twenty years of antimicrobial resistance control programme in a regional multi hospital institution, with focus on emerging bacteria (VRE and CPE). *Antimicrob. Resistance Infect. Control* 1:1-4.
- Gabriel I, Alleman F, Dufourcq V, Perrin Q, Gabarrou JF (2013). Utilisation des huiles essentielles en alimentation des volailles.2; Hypothèses sur les modes d'action impliqués dans les effets observés. *INRA Prod. Anim.* 26(1):13-24.
- Haddouchi F, Lazouni HA, Meziane A, Benmansour A (2009). Etude physicochimique et microbiologique de l'huile essentielle de *Thymus fontanesii* Boiss & Reut. *Afr. Sci.* 4(2):246-259.
- Hilan C, Bouaoun D, Aoun J, Sfeir R, Garabeth F (2009). Propriétés antimicrobiennes et toxicité par détermination de la DL50 de l'huile essentielle de *Prangos asperula* Boissier. *Phytothérapie* 7:8-14.
- Jing-yi Zhao ZYQ, Li YN, Mu XD, You LP, Xu C, Qin P, Ma JI (2014). Coexistence of SFO-1 and NDM-1 β -lactamase genes and fosfomycin resistance gene fosA3 in an *Escherichia coli* clinical isolate. *FEMS Microbiol. Lett.* 362:1-7.
- Kalembe D, Kunicka A (2003). Antibacterial and antifungal properties of essential oils. *Curr. Med. Chem.* 10:813-829.
- Kassi MF, Badou JO, Tonzibo FZ, Salah Z, Bolou A, Camara B, Kone D (2014). Potentiel antifongique de l'huile essentielle de *Ocimum gratissimum* dans la lutte biologique contre les maladies des raies noires du bananier causées par *Mycosphaerella fijiensis* Morelet (Mycosphaerellaceae). *Agron. Afr.* 26(2):1-11.
- Kheyar N, Meridja D, Belhamel K (2014). Etude de l'activité antibactérienne des huiles essentielles d'*Inula viscosa*, *Salvia officinalis* et *Laurus nobilis* de la région de Bejaia. *Algerian J. Nat. Prod.* 2(1):18-26.

- Koba K, Sanda K, Guyon C, Raynaud C, Chaumont JP, Nicod L (2009). In vitro cytotoxic activity of *Cymbopogon citratus* L. and *Cymbopogon nardus* L. essential oils from Togo. J. Bangladesh Pharm. Soc. (BDPS) 4:29-34.
- Koba K, Sanda K, Raynaud C, Nenonene YA, Millet J, Chaumont J (2004). Activités antimicrobiennes d'huiles essentielles de trois *Cymbopogon* sp. africains vis-à-vis de germes pathogènes d'animaux de compagnie. Ann. Méd. Vét. 148:202-206.
- Kone D, Camara B, Badou Odjoutchoni J, Doumbouya M, Soro S, N'Guessan Aya C, Bomisso EL (2010). Fungicides and biological products activities towards fungi causing diseases in Banana and Vegetable in Côte d'Ivoire. Dans O. Carisse (Éd.), *Fungicides*. In Tech. pp. 39-68.
- Kouamé KG, Kouassi KI, Kassi FM, Bolou Bi Bolou A, Tuo S, Kanko C, Kone D (2015). Antifungal Activity of Essential Oils Extracted from *Monodora myristica* (Gaertn), *Ocimum gratissimum* L. and *Zingiber officinalis* Roscoe on Post-harvest Anthracnose of Mango Fruit (*Mangifera indica* L.) Variety Kent in Côte d'Ivoire. Int. J. Sci. 4(12):8-18.
- Kpodekon MT, Boko KC, Mainil JG, Farougou S, Sessou P, Yehouenou B, Bardiau M (2013). Composition chimique et test d'efficacité in vitro des huiles essentielles extraites de feuilles fraîches du basilic commun (*Ocimum basilicum*) et du basilic tropical (*Ocimum gratissimum*) sur *Salmonella enterica* sérotypes Oakland et Legon. J. Soc. Ouest-Africaine Chimie 35:41-48.
- Li D, Wu C, Wang Y, Fan R, Schwarz S, Zhang S (2015). Identification of Multiresistance Gene *cf*r in Methicillin-Resistant *Staphylococcus aureus* from Pigs: Plasmid Location and Integration into a Staphylococcal Cassette Chromosome *mec* Complex. Antimicrob. Agents Chemother. 59:3641-3644.
- Mahanta JJ, Chutia M, Bordoloi M, Pathak M, Adhikary R, Sarma TC (2007). *Cymbopogon citratus* L. essential oil as a potential antifungal agent against key weed moulds of *Pleurotus* spp. spawns. Flavour Fragrance J. 22:525-530.
- Nakamura CV, Ueda-Nakamura T, Bando E, Abrahão Fernandes, NM, Cortez DA, Dias Filho BP (1999). Antibacterial Activity of *Ocimum gratissimum* L. Essential Oil. Memórias Instituto Oswaldo Cruz, Rio de Janeiro 94(5):675-678.
- Ngom S, Diop M, Mbengue M, Faye F, Kornprobst JM, Samb A (2014). Composition chimique et propriétés antibactériennes des huiles essentielles d'*Ocimum basilicum* et d'*Hyptis suaveolens* (L.) Poit récoltés dans la région de Dakar au Sénégal. Afr. Sci. 10(4):109-117.
- Soro LC, Grosmaire L, Ocho-Anin Atchibri AL, Sylvie M, Menut C, Pelissier Y (2015). Variabilité de la composition chimique de l'huile essentielle des feuilles de *Lippia multiflora* cultivées en Côte d'Ivoire. J. Appl. Biosci. 88:8180-8193.
- Tibyangye J, Okech MA, Nyabayo JM, Lukanga Nakavuma J (2015). In vitro Antibacterial Activity of *Ocimum suave* Essential Oils against Uropathogens Isolated from Patients in Selected Hospitals in Bushenyi District, Uganda. Br. Microbiol. Res. J. 8(3):489-498.
- Traoré N, Sidibe L, Bouare S, Harama D, Somboro A, Fofana B, Chalchat JC (2013). Activités antimicrobiennes des huiles essentielles de *Eucalyptus citriodora* Hook et *Eucalyptus houseana* W.Fitzg. ex Maiden. Int. J. Biol. Chem. Sci. 7(2):800-804.
- Yang XN, Imran K, Sun CK (2015). Chemical composition, mechanism of antibacterial action and antioxidant activity of leaf essential oil. Asian Pac. J. Trop. Med. 8(9):694-700.

Full Length Research Paper

Acute toxicity study of *Sphaeranthus bullatus* used as herbal tea in Tanzania

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Received 19 September, 2016; Accepted 11 November, 2016

***Sphaeranthus bullatus* aerial part is used by Chaga people in Northern Tanzania as herbal tea. It is usually drunk for the management of diarrhea. In this study acute toxicity of *S. bullatus* was evaluated in order to establish its safety to verify its use as herbal tea. Acute toxicity was performed per Organization for Economic co-operation and Development 423 (OECD) guideline. Six groups of female white albino mice were orally administered as a single dose of 1000, 2000 mg/kg body weight, 3000, 4000 and 5000 mg/kg body weight of *S. bullatus* dichloromethane extract. Internal organ weights, hematological and biochemical parameters of animal blood were determined after 14 days of the experiment. No animal died up to the 14th day of the experiment. Red blood cells, haemoglobin concentration, and hematocrit concentration of the treated animals were significantly higher than the control while white blood cells and platelets were found lower than the control animals. Level of biochemical parameters of the treated animals were not significantly different from the control animals except the level of bilirubin of treated animals which was found higher than control animals. *S. bullatus* dichloromethane extract is therefore established to have safety up to dose of 5000 mg/kg body weight which authenticates its use as herbal tea and for management of diarrhea. However a dose of more than 2000 mg/Kg body weight indicated to lower WBC.**

Key words: *Sphaeranthus bullatus*, herbal tea, physical observation, hematology, biochemical.

INTRODUCTION

Herbs have been used to treat ailments for ages (Motaleb et al., 2011). Information on their use passed from generation to generation (Nwachukwu et al., 2010). Recently there has been a growing trend of using infusions or decoction of plant parts to make herbal tea;

they are mainly used to treat ailments but also as a healthy alternative to caffeinated beverages such as coffee, tea and cocoa (Shaw et al., 2012; Mainteiga et al., 1997; Marete et al., 2011). One of these plants used in Tanzania for this purpose is *Sphaeranthus bullatus*

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(Asteraceae).

Herbal tea consisting of *S. bullatus* infusions is being used in Northern Tanzania to manage diarrhea; *S. bullatus* is an East African native plant found in wet areas along streams and swamp sites (Machumi et al., 2012). Apart from being used as herbal tea concoction of its leaves are used to treat stomach problems, astheniam and malaria in East Africa (Quantrocchi, 2006; Machumi et al., 2102). It is also used to remove retained placenta to livestock in Tanzania and Kenya (Quantrocchi, 2006). *S. bullatus* contain carvotacetone derivatives which usually have antibacterial activities (Machumi et al., 2012). Moreover, dichloromethane extracts of *S. bullatus* exhibited higher antibacterial activity in the study conducted by Ochanga and Chacha (unpublished paper). However, its acute toxicity has never been done.

In order to establish its safety, *S. bullatus* dichloromethane extract which exhibited higher antibacterial activity was evaluated for acute toxicity. Acute toxicity test has been established as the effective method of testing effects caused by exposure of harmful substances and the tested animals are usually monitored within 14 days of administration of the substance. However, acute toxicity occurs over a very short period of time, usually within 24 h (OECD, 2000).

MATERIALS AND METHODS

Aerial parts of *S. bullatus* were collected from Tengeru in Arumeru districts. The plant parts were identified by trained taxonomist working in the National herbarium, Tropical Pesticide Research Institute (TPRI), Arusha, Tanzania. Voucher specimen was deposited at Nelson Mandela African institution of Science and Technology (NM-AIST), Arusha Tanzania.

Preparation and extraction of plant material

Collected plant sample was air dried under the shade and pulverized. The pulverized material (250 g) was sequentially macerated using dichloromethane for 48 h. The mixtures was filtered through cotton wool then with filter paper No. 1 and dichloromethane was removed through vacuum using rotary evaporator and stored at -20°C until the time of testing.

Experimental animals

A total of 18 female Swiss albino mice (24 to 41 g) were obtained from the Department of Agriculture of the Tropical Pesticide Research Institute (TPRI) in Arusha, Tanzania. Female mice were selected because they are known to be slightly more sensitivity to phytochemical than male (OECD, 2001). Animals were housed in a cage in a well ventilated room and maintained with light for 12 h and darkness for 12 h. They were fed on diet prepared from chick Grower's mash (BCA Grain and Feed Company Limited) and were given water *ad libitum* throughout the study period. Acute toxicity was performed as per organization for Economic Co-operation and Development (OECD) guidelines 423 (OECD, 2001) and was in compliance with the procedure of National Health Research Ethics Sub-Committee of the National Institute for Medical Research of Tanzania (permit number No. NMR/HQ/R.8a/Vol. IX 2163).

Experimental design for acute toxicity

Mice were assigned to six groups of three mice. Group 1 mice were given 1000 mg/Kg body weight, group 2 were given 2000 mg/Kg body weight, group 3 were given 3000 mg/ Kg body weight, group 4 were given 4000 mg/Kg body weight and group 5 were given 5000 mg/Kg body weight of the extracts. Group six were set as control given only feeds and water. All experimental animals were assigned special identification and were exposed to feeds and water in an *ad libitum*. These extracts were orally given as a single dose after 6 days of adaptation to the room environments.

Mode of administration

The albino mice were fasted on water for 4 h, and then weighed. They were then administered a single dose consisting of 5 ml of plant extract dissolve/mixed in polysorbate -80(tween 80). The amount of extract in 5 ml was given in accordance to the weight of each mouse, calculated as follows:

$$\text{Administration volume} = \frac{5\text{mL} \times \text{weight of mouse (g)}}{1000 \text{ g}}$$

Food was given to the mice three hours after administering test extract.

Physical observation

Animals were observed at least after every 30 min during the first 24 h with special attention given to the first 10 h. Observation and recording of any sign of illness continued three times a day for 14 days. Observations of signs like eyelid closure, difference in breathing, change of skin and fur, eye muscle membranes, sleep, general weakness, loss of appetite, diarrhea, change of body weight and mortality.

Hematological and biochemical parameters

At the end of 14th day of the observation of physical parameters animals were sacrificed for hematological and biochemical parameters studies. Briefly animals were sacrificed by restraining them using chloroform in desiccators jar, blood was collected from the experimental animals by cardiac puncture using sterile needle and 5 ml syringe. Blood aliquot was immediately transferred into ethylene-diamine-tetra-acetic acid (EDTA) bottles to prevent coagulation factors in cell-cell and cell-matrix interactions for hematological determinations. Other portion of blood were left in the syringe without EDTA and allowed to clot for biochemical investigation. Clotted blood was centrifuged for 5000 revolution per minute for five minutes to obtain serum for biochemical studies. Red blood cell count (RBC), hemoglobin concentration, hemacrotic (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC), absolute content of lymphocyte (LMP), absolute content of neutrophils (NEUT), proportion of whole blood occupied by platelets (PCT), the relative width of the distribution in volume index of the heterogeneity of platelets (PDW), relative distribution width of red blood cell by volume- standard deviation (RDW-SD), relative distribution width of red blood cell by volume, coefficient of variation (RDW-CV), ratio of large platelets (P-LRC), absolute content of monocyte (MONO), absolute content of eosinophils (EO), and absolute content of basophil (BASO) hematological parameters were analyzed. Moreover, biochemical parameters such as alanine

aminotrans liquid, aspartate aminotrans liquid, albumin, total protein, cholesterol, triglycerides liquid, and bilirubin were analyzed for biochemical blood investigation.

Observation of internal organs and calculation of organ - body weight index

Experimental animals were sacrificed using chloroform in the desiccators. Gross observation of the liver, heart, spleen, and kidney were done for treated and control animals to observe any sign of abnormalities. However the weight of animal at the 14th day and weight of organs after dissection were taken and used to calculate organ-body weight index.

$$\text{Organ - body weight index} = \frac{\text{Organ weight (g)}}{\text{Body weight (g)}} \times 100$$

Statistical analysis

Statistical analysis was performed using Statistical. Ink version 8 computer program. Result was presented as Mean \pm Standard deviation of mean (SD). One way analysis of variance (ANOVA) was employed for between and within group variation. To compare two variable students T-test was employed. Post hoc (TURKEY HSD) analysis was used to show where the variation occurs. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Physical observation

S. bullatus dichloromethane extract (SBDE) was evaluated for acute toxicity study as per OECD guideline 423. Physical observation of the mice revealed that there was neither mortality nor signs of toxicity in all animals up to 14th day after administering SBDE up to 5000 mg/Kg body weight. It was also observed that the body weights of both treated and control mice increased significantly (Table 1) from the day zero to day 14 ($P < 0.05$). Moreover, there was no significant difference in the change of weights in all mice given doses from 1000 mg/Kg body weight to 5000 mg /Kg body weight and control mice between the first day of administration of plant extract and 14th day ($P > 0.05$). Since none of the SBDE tested dosage induced mortality or any sign of toxicity, it is therefore suggestive that SBDE is non toxic to a dosage of 5000 mg/Kg body weight which is supported by Kabubii et al. (2015).

Macroscopic examination of internal organs

The macroscopic examination of the internal organs of the mice revealed no differences between the control and the treated mice. In toxicology studies internal organs such as livers, heart, lungs, spleen and kidney are primarily affected by metabolic reaction caused by toxicants (Akindele et al., 2014).

Organ body weight index

Difference in organ weights between treated and untreated mice has widely been accepted to assess toxic effects of foreign substance(s) in the organs (Michael et al., 2007; Nirogi et al., 2013; Piao et al., 2013). In this study, liver, heart, lung, kidney and spleen were considered as recommended for toxicological studies (Akindele et al., 2014). Although there was a variation in organ body weight index between most of organs of mice tested with that of control mice as shown in Table 2, variation was not significant except for the liver of mice given 5000 mg/Kg body weight of the extract ($P > 0.05$, Turkey HSD).

Hematological parameters

The hematological parameters test is used to reveal the adverse effect of the extract in the blood constituents of the animal or determine possible alteration in the level of enzymes, metabolic products and normal functioning of organs (Michael et al., 2007). Results obtained from this study indicated that white blood cells (WBC) count or leukocytes of both treated and untreated mice were in the normal health range ($3.20 - 8.20 \times 10^9/L$). However WBC of mice given 3000 to 5000 mg/Kg body weight were significantly lower ($P < 0.05$) than the control mice (Table 3). In this study, neutrophils, lymphocytes, eosinophils, monocytes, basophils were evaluated to establish any variation between the tested and control groups. Among these significance variations were observed in neutrophil. The function of neutrophil in the body of animals is to digest foreign substances (Kaplan, 2013). Low level of WBC can be caused by number of conditions including presence of anticancer drugs in the body (Cancer Care, 2014). Anticancer activity of *S. bullatus* has been reported by Machumi et al. (2012). This study suggests that the gradual decrease of the level of WBC was probably contributed by presence of *S. bullatus* in the body of animal.

Blood sample from experimental and control mice were analyzed for the red blood cell count (RBC), haemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width (RDW) (Akindele et al., 2014). Results from this analysis are shown in Table 3. The RBC, HGB and HCT of the treated mice were significantly higher than that of the control mice ($P < 0.05$). However, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width (RDW) of the treated animals were not significantly different from the control animals ($P > 0.05$) indicating signs of absence of anemia causing compounds in SBDE (Kabubii et al., 2015).

Platelets parameters namely platelets count (PLT),

Table 1. Effect of dichloromethane extract of *S. bullatus* on the body weight of white albino mice given as mean and standard deviation.

Groups	Dose (Mg/Kg bwt)	Day 0 wt (g)	Day 14 wt (g)
I	1000	23.67 ± 1.15	26.33± 1.53
II	2000	30.33 ± 2.08	32.67± 2.31
III	3000	27.67 ± 2.52	30.67± 1.52
IV	4000	31.33 ± 1.15	33.33± 0.58
V	5000	40.33 ± 1.53	41.67± 1.53
VI	Control	24.67 ± 0.58	27.33± 1.15

Bwt, Body weight, wt, weight.

Table 2. Effect of dichloromethane extracts of *S. bullatus* on organ - body weight index of white albino mice given as mean and standard deviation.

Organ	1000	2000	3000	4000	5000	Control
Liver	8.45 ± 1.18	8.02 ± 0.07	7.40 ± 0.43	7.71 ± 0.40	9.27 ± 0.27*	7.23 ± 0.46
Heart	0.57 ± 0.09	0.56 ± 0.12	0.50 ± 0.07	0.59 ± 0.00	0.62 ± 0.04	0.61 ± 0.02
Lung	0.91 ± 0.13	0.98 ± 0.11	0.95 ± 0.04	1.00 ± 0.04	0.91 ± 0.01	0.70 ± 0.03
Spleen	0.35 ± 0.02	0.67 ± 0.25	0.55 ± 0.04	0.50 ± 0.01	0.59 ± 0.05	0.49 ± 0.07
Kidney	0.70 ± 0.07	0.87 ± 0.06	0.66 ± 0.07	0.65 ± 0.03	0.79 ± 0.01	0.70 ± 0.03

Organ, body weight index = organ weight/body weight x 1000; * Significant different difference in row.

mean platelet volume (MPV), the ratio width of distribution of platelets in volume index of heterogeneity of platelets (PDW) and proportion of whole blood occupied by platelets (PCT) were analyzed. Platelets are important as thrombosis mediator which stop bleeding and to accelerate vascular inflammatory conditions in the body (Morrel et al., 2014). It was found that PLT of the treated mice was significantly lower ($P < 0.05$) than the control mice (Table 3). Other parameters of the platelets measurements such as mean platelet volume (MPV), the ratio width of distribution of platelets in volume index of heterogeneity of platelets (PDW) and proportion of whole blood occupied by platelets (PCT) of the treated animals were not significantly different from the control animals except platelets large cell ratio (P-LCR) of the treated mice was found to be significantly higher than the control mice ($P < 0.05$).

Biochemical parameters

Enzymes that play major roles in the metabolism and detoxification of compounds in the liver namely alanine aminotranse (ALT) and aspartate Aminotranse (AST) were considered to establish if there was cellular damage in the liver (Shah and Sass, 2015). The increase in the concentration of ALT and AST in the blood indicates liver injury (Velayudam et al., 2013). In this study there was no significant difference in the level of ALT and AST in treated and untreated animals (Table 4), which indicates that SBDE is not harmful to the liver (Velayudam et

al., 2013).

The total protein level was analyzed and the findings indicated that both treated and control mice had normal protein level of 75.0 g/L (Table 4). The normal protein level range from 63.0 to 82.0 g/L, thus the total protein level of the analyzed blood falls within the normal range (Agbaje et al., 2009). It is therefore conclusive that SBDE did not induce inflammation to the body of mice treated (Fakanya and Tothil, 2014). Erythrocyte sedimentation rate, C-reactive protein and plasma viscosity are blood tests that detect inflammation (Fakanya and Tothil, 2014). It has been established that extra protein released in the blood stream usually from the site of inflammation is used as a marker of inflammation (Chan et al., 2002).

Cholesterol and triglycerides level in the blood of experimental and control mice were determined to establish if SBDE elevates level of aforementioned compounds. Level of cholesterol and triglyceride in the body of treated albino mice and untreated ones was not significantly different ($P < 0.05$) which suggests that SBDE doesn't elevate the level of cholesterol and fatty acids. High level of cholesterol and triglycerides emanating from diet has implicated to cause heart diseases and stroke (Sher et al., 2012).

In this study, SBDE was assessed if its use could lead to destruction of blood cells. It was monitored by the determination of the level of bilirubin in blood. The breakdown of blood cells is usually done in the liver and bilirubins are removed out of the body through defecation (Hansen, 2010). High level of bilirubin in the body is usually implicated by liver failure to remove them from the

Table 3. Effect of dichloromethane extracts of *S. bullatus* on hematological parameters of white albino mice.

Parameter	Control	1000 mg/Kgbwt	2000 mg/Kgbwt	3000 mg/Kgbwt	4000 mg/Kgbwt	5000 mg/kg bwt
RBC (10 ⁹ /L)	4.77 ± 0.11	5.17 ± 0.13	5.35 ± 0.08	5.63 ± 0.10	5.79 ± 0.05	5.92 ± 0.03
HGB (g/dL)	13.70 ± 0.26	13.91 ± 0.48	14.13 ± 0.1	14.13 ± 0.31	14.37 ± 0.22	14.40 ± 0.20
HCT (%)	39.87 ± 0.49	40.09 ± 0.71	41.98 ± 0.67	40.95 ± 0.87	42.11 ± 0.65	42.53 ± 0.57
MCV (fL)	83.07 ± 0.46	83.74 ± 0.36	81.58 ± 0.77	81.49 ± 0.59	81.53 ± 0.17	81.46 ± 0.23
MCH (pg)	29.76 ± 0.47	29.12 ± 0.79	29.19 ± 0.14	29.19 ± 0.18	29.24 ± 0.73	29.27 ± 0.12
MCHC (g/dL)	35.10 ± 0.10	35.18 ± 0.17	35.70 ± 0.10	35.61 ± 0.14	35.53 ± 0.19	35.63 ± 0.12
RDW-SD (fL)	43.00 ± 0.1	43.01 ± 0.58	43.03 ± 0.82	42.45 ± 0.36	42.89 ± 0.97	42.73 ± 0.45
RDW-CV (%)	14.47 ± 0.12	15.13 ± 0.54	15.45 ± 0.29	15.32 ± 0.59	15.45 ± 0.63	15.53 ± 0.47
PLT (10 ⁹ /uL)	243.00 ± 2.0	193 ± 2.28	193 ± 2.15	193 ± 2.66	194.19 ± 2.14	194.33 ± 2.52
PDW (fL)	10.37 ± 1.01	9.87 ± 0.92	9.63 ± 0.03	9.63 ± 0.04	9.63 ± 0.02	9.63 ± 0.40
MPV (fL)	9.30 ± 0.26	9.30 ± 0.19	9.49 ± 0.20	9.83 ± 0.43	9.78 ± 0.38	9.97 ± 0.40
P-LCR (%)	18.97 ± 0.51	20.91 ± 0.32	21.18 ± 0.37	23.19 ± 0.54	23.63 ± 0.31	24.43 ± 1.36
PCT (%)	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.03	0.23 ± 0.04	0.23 ± 0.03	0.21 ± 0.02
WBC (10 ⁹ /L)	5.29 ± 0.03	5.27 ± 0.08	5.26 ± 0.11	4.76 ± 0.21	4.42 ± 0.13	4.47 ± 0.28
NEUT (10 ⁹ /L)	3.33 ± 0.12	3.31 ± 0.23	3.30 ± 0.12	2.27 ± 0.25	2.28 ± 0.37	2.26 ± 0.12
LYMP (10 ⁹ /L)	1.69 ± 0.12	1.72 ± 0.16	1.74 ± 0.19	1.78 ± 0.14	1.82 ± 0.25	1.87 ± 0.06
MONO (10 ⁹ /L)	0.43 ± 0.01	0.42 ± 0.77	0.42 ± 0.03	0.42 ± 0.06	0.42 ± 0.07	0.42 ± 0.02
OE (10 ⁹ /L)	0.10 ± 0.00	0.21 ± 0.06	0.22 ± 0.03	0.22 ± 0.54	0.21 ± 0.01	0.22 ± 0.03
BASO (10 ⁹ /L)	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01

Bwt, Body weight

Table 4. Biochemical parameters.

Parameter	Control	1000 mg/Kgbwt	2000 mg/kgbwt	3000 mg/Kgbwt	4000 mg/Kgbwt	5000 mg/kg bwt
ALT (U/L)	28.83 ± 0.35	29.17 ± 0.52	29.61 ± 0.76	29.63 ± 0.49	30.41 ± 0.68	30.57 ± 1.36
AST (U/L)	42.00 ± 0.41	42.04 ± 0.35	42.13 ± 0.33	42.35 ± 0.37	42.35 ± 0.33	41.37 ± 0.31
Albumin Gen 2. (g/L)	51.00 ± 1.91	52.37 ± 1.80	52.40 ± 1.87	52.81 ± 1.95	52.82 ± 1.97	52.83 ± 2.29
Total protein (g/L)	75.33 ± 0.31	75.35 ± 0.32	75.35 ± 0.33	75.39 ± 0.33	75.40 ± 0.35	75.40 ± 0.35
Cholesterol (mmol/L)	4.48 ± 0.03	4.48 ± 0.03	4.48 ± 0.03	4.48 ± 0.03	4.48 ± 0.03	4.48 ± 0.03
Triglycerides liquid (mmol/L)	1.49 ± 0.12	1.49 ± 0.19	1.49 ± 0.11	1.49 ± 0.21	1.49 ± 0.20	1.49 ± 0.07
Bilirubin (Umol/L)	1.26 ± 0.02	1.51 ± 0.01	1.51 ± 0.00	1.52 ± 0.03	1.52 ± 0.01	1.53 ± 0.01

Bwt, Body weight.

blood stream leads to health condition known as jaundice. The present study revealed that the treated animals have high significant level of bilirubin compared to untreated animal ($p < 0.05$). This is probably due to breakdown of cells caused by compounds in *S. bullatus* with anticancer activity.

Conclusion

S. bullatus dichloromethane extract has been established to have safety up to dose of 5000 mg/Kg body weight which authenticates its use as herbal tea and for management of diarrhea. However a dose more than 2000 mg/Kg body weight indicated to lower WBC.

ACKNOWLEDGEMENT

This work was funded by Government of Tanzania through the Nelson Mandela African Institution of Science and Technology. The authors would like to acknowledge Daniel Sitoni from TPRI for the identification of plant species used in this study.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Agbaje EO, Adeneye AA, Daramola AO (2009). Biochemical and

- toxicological studies of aqueous extract of *Syzygium aromaticum* (L.) Merr. & Perry (Myrtaceae) in rodents. *Afr. J. Tradit. Complement. Altern. Med.* 6(3):241-254.
- Akindede AJ, Adeneye AA, Salau OS, Sofidiya MO, Benebo AS (2014). Dose and time-dependent sub-chronic toxicity study of hydroethanolic leaf extract of *Flabellaria paniculata* Cav. (Malpighiaceae) in rodents. *Front Pharmacol.* 5:78.
- Cancer care (2014). Neutropia (low white blood cell) cancer care Nova Scotia. 1-866-599-2267).
- Chan YL, Liao HC, Tsy PK, Chang SS, Chen JC, Liaw SJ (2002). C-reactive protein as indicator of bacterial infection of adult patients in the emergency department. *Chang Gung Med. J.* 25(7):437-445.
- Fakanya WM, Tothil IE (2014). Detection of inflammation biomarkers C-reative protein in serum samples towards an optimal biosensor. *Biosensors (Basel)* 4(4):340-357.
- Hansen TWR (2010). Core concept: bilirubin metabolism. *Neo Review.* 11(6):316-322.
- Kabubii ZN, Mbaria J, Mathiu M (2015). Acute toxicity studies of Myrsine Africana aqueous seed extract in male Wistar rats on some hematological and biochemical parameters. *Clin. Phytosci.* 1:9.
- Kaplan MJ (2013). Role of neutrophils in systemic autoimmune diseases. *Arthritis Res. Ther.* 15:219.
- Machumi F, Yenesewa A, Midiwoa JO, Heydenreichb M, Kleinpeterb E, Tekwanic BL, Khan SI, Walker LA and Muhammad I (2012). Antiparasitic and anticancer carvotacetone derivatives of *Spheranthusbullatus*. *Nat Prod. Commun.* 7(99):1123-1126.
- Marete EN, Jacquier JC, O'Riordan D (2011). Feverfew as a source of bioactive for functional foods: Storage stability in model beverages. *J. Funct. Foods* 3:38-43.
- Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, Johnson JK, Schafer K (2007). Evaluation of organ weights for rodent and nonrodent toxicity studies: A review of Regulatory Guidelines and a Survey of Current Practices. *Toxicol. Pathol.* 35:742-750.
- Morrel CN, Aggrey AA, Chapman LM, Modjeski KM (2014). Emerging roles for platelets as immune and inflammatory cells. *Blood* 123(18):2759-2767.
- Nirogi R, Goyal VK, Jana S, Pandey SK, Goth A (2014). What suits best for organ weight analysis: Review of relationship between organ weight and body/brain weight for rodent toxicity studies. *Int. J. Pharm. Sci. Res.* 5(4):1525-1532.
- Nwachukwu CU, Umeh CN, Kalu IG, Slyvester O, Magnus N (2010). Identification and Traditional uses of some common medicinal plants in Ezinihitte Mbaise LGA of Imo State, Nigeria. *Report Opin.* 2(6):1-8.
- Organization for Economic Co-operation and Development (OECD) (2001). Guidelines for the Testing of Chemicals "Acute Oral Toxicity-Acute Toxic Class Method" No. 423
- Organization for Economic Co-operation and Development (OECD) (2000). Guidance document on acute oral toxicity. Environmental health and safety monograph Series on testing and assessment: No 24.
- Piao Y, Liu Y, Xie X (2013). Change Trends of Organ Weight Background Data in Sprague Dawley Rats at Different Ages. *J. Toxicol Pathol.* 26(1):29-34.
- Quantrocchi U (2012). CRC world dictionary of medicinal and poisonus plants: common names, scientific names, eponyms, synonymis and entomology. CRC press. 5:3960p.
- Shah SC, Sass DA (2015). Cardiac Hepatopathy: A review of liver dysfunction in heart failure. *Liver Res. Open J.* 1(1):1-10.
- Shaw YAB, Salmon CNA, Green CE, Hibbert SL, Smith AM, and Williams LAD (2012). Evaluation of the Nutraceutical Potential of *Rytidophyllum tomentosum*(L.) Mart.: HPTLC Fingerprinting, Elemental Composition, Phenolic Content and *in vitro* Antioxidant Activity. *Pharm. Crops.* 3(47):2210-2906.
- Sher A, Mahmood MFU, Shah SNH, Bukhsh S, Murtaza G (2012). Effect of garlic extract on blood glucose level and lipid profile in normal and alloxan diabetic rabbits. *Adv. Clin. Exp. Med.* 21(6):705-711.
- VElayudam, Ilavarasan, Arul amuthan (2013). Acute and28-day subchronic oral toxicity study of kadukkai maathirai, an iron based siddha herbal formulation in wistar albino rats. *Int. J. Pharm. Pharm. Sci.* 5(4):186-191.

The background of the entire page is a close-up photograph of a wooden mortar and pestle. The mortar is filled with a vibrant green, finely ground powder. The pestle is positioned vertically to the right of the mortar. The wooden surface has a rich, dark brown grain. The text is overlaid on this image.

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