

In vitro antibacterial activity of two medicinal plants used in Bénin to treat microbial infections

Lagnika L^{1*}, Amoussa AMO², Sanni A³

1. Lecturer, researcher, Dept. of Biochem and Cell. Biol., Laboratory of Biochemistry and bioactive natural substances, University of Abomey-Calavi, 04 BP 0320, Cotonou, Bénin, West Africa
2. PhD, Dept. of Biochem and Cell. Biol., Lab. of Biochem. and bio. Nat. Subst., Univ. of Abomey-Calavi, 04 BP 0320, Cotonou, Bénin, West Africa
3. Senior Lecturer, Dept. of Biochem and Cell. Biol., Lab. of Biochem. and bio. Nat. Subst., Univ. of Abomey-Calavi, 04 BP 0320, Cotonou, Bénin, West Africa

*Corresponding author: Lecturer, researcher, Univ. of Abomey-Calavi, 04 BP 0320, Cotonou, Bénin, West Africa, Mail: latifkabe@yahoo.fr; Mobile No: (+229) 97604889

Publication History

Received: 06 December 2013

Accepted: 23 January 2014

Published: 5 February 2014

Citation

Lagnika L, Amoussa AMO, Sanni A. In vitro antibacterial activity of two medicinal plants used in Bénin to treat microbial infections. *Indian Journal of Science*, 2014, 8(19), 10-15

ABSTRACT

Dichloromethane, methanol and hydroethanol extracts of *Casuarina equisetifolia* L. and *Oxalis corniculata* L., two medicinal plants used by traditional doctors to treat microbial infections, were screened for their antibacterial activity against seven Gram positive and Gram negative bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Salmonella aboni*, *Staphylococcus aureus* methicillin resistant and *Staphylococcus epidermidis*). The antibacterial activity was performed using the *p*-iodonitrotetrazolium microdilution method. The total activity and *Artemia salina* lethality of extracts were also determined. All extracts were effective against tested microorganisms at different levels with Minimum Inhibitory Concentration values ranging from 0.078 mg/ml to 5 mg/ml. The hydroethanolic extract was more potent than other extracts with a MIC value of 0.078 mg/ml against *S. epidermidis*. The most interesting total activity was obtained with hydroethanolic extract of *Oxalis corniculata* (1689.7 ml). The methanol extract of *Oxalis corniculata* was also the less toxic to *Artemia salina* with LC₅₀ value of 26.87 mg/ml.

Keywords: Medicinal plants, antibacterial, toxicity, *artemia salina*

1. INTRODUCTION

Infectious diseases are becoming a crisis as a major cause of human and animal mortality and morbidity. This is further aggravated by the rapid development of multi-drug resistance, limited antimicrobial spectrum and adverse

Lagnika et al.

In vitro antibacterial activity of two medicinal plants used in Bénin to treat microbial infections, *Indian Journal of Science*, 2014, 8(19), 10-15, <http://www.discovery.org.in/ijs.htm>

Comparison: This is to check phased manner, the accuracy of the data related to the use of a product, using methods approved by science. This audit will confirm or refute empirical data taken by a segment of the population, based on the results.

Content: Verification of data and past practices. This will be recorded in documents such as articles, books, etc., or disseminated through the press.

Iodonitrotetrazolium: It is an artificial electron acceptor which can be used in a colorimetric assay to determine the presence of protein in culture median. The presence of protein such as threonine deshydrogenase during active growth of bacteria changes the color of INT (incolore) to purple-red.

Muller Hinton: It is a microbiological growth median that is commonly used for antibiotic, extracts or natural compounds susceptibility testing.

effects of available anti-microbial agents (Doughart and Okafor, 2007). In most countries of West Africa, the advent of modern medicine and its advances have led people to shy away some traditional medicine, based primarily on herbal medicine. Unfortunately, development of antibacterial agents has been accompanied by the emergence of drug-resistant organisms followed by toxicity observed during prolonged treatment. The use of plants for healing dates from prehistoric times and all peoples have this old tradition. The use of herbs in the treatment of man and animal disease has been also practiced before the advent of modern antibiotics (George, 1974; Soforowa, 1982). Thus, in recent decades, the use of medicinal plants has been renewed interest. Special attention is given to the search for new and effective pharmaceutical agents, with little or no toxicity, from medicinal plants. Natural products and related structures are essential sources of new pharmaceuticals, because of the immense variety of functionally relevant secondary metabolites of microbial and plant species (Ngo et al. 2013). Approximately half of all drugs that were recorded worldwide in the period before 2007 were from natural products or their synthetic derivatives (Kennedy and Wightman, 2011). Several scientific studies have confirmed the activities of most of the plants used in traditional medicine not only against microbial infections (Traoré et al. 2012; Bolou et al. 2011; Adedapo et al. 2009). In Benin, plants materials have been used as traditional medicines for the treatment of a wide variety of ailments and diseases. *Casuarina equisetifolia* and *Oxalis corniculata* are two plants commonly used in Benin traditionally medicine against infections of the skin, dizziness, diarrhea, stomach ache, dysentery, convulsions and other digestive problems.

2. SCOPE OF THE STUDY

The present work is to investigate the antibacterial properties of *Casuarina equisetifolia* and *Oxalis corniculata* against six strains of bacteria *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Staphylococcus aureus* *meticilline* resisting. The lethality effect of extracts was also evaluated using *Artemia salina*.

2.1. Materials

The materials used include fruits of *Casuarina equisetifolia*, leaves of *Oxalis corniculata* and others which include, pipette, bottles, weighting balance, conical flasks, petri dishes, and electric blender, rotary evaporator, hood, oven, mixer etc.

2.1.1. Reagent/chemical/median

p-iodonitrotetrazolium (Sigma Aldrich) solution was prepared by dissolving 4 mg in 200 ml distilled water. The Muller Hinton broth (DIFCO) was prepared by dissolving 21 g in 1 L distilled water. The suspension was then sterilized using autoclave.

2.1.2. Organisms used

Escherichia coli (CIP53126), *Staphylococcus aureus* (ATCC6538), *Enterococcus faecalis* (ATCC29212), *Pseudomonas aeruginosa* (CIP82118), *Salmonella aboni* (CIP8039), *Staphylococcus aureus* *meticilline* resisting (SARM) and *Staphylococcus epidermidis*, obtained from Laboratoire de Biophotonique et Pharmacologie, University of Strasbourg, France, were used for antibacterial activity. Eggs of *Artemia salina* were hatched in seawater to evaluate extracts toxicity.

2.2. Methodology

2.2.1. Preparation of extracts

One hundred grams (100 g) of dry powder of each part of the two plants were successively extracted by maceration with dichloromethane and methanol for 72 h stirring. A second maceration with a mixture of ethanol-water (20:80) was carried out with fifty grams (50 g) of dry powder species. Each extraction is repeated three times. The macerates were filtered and concentrated using a rotary evaporator (RE 300, stuart) and the extracts were stored at 4° C until biological assay.

2.2.2. Antibacterial activity

Growth inhibitory effect of extracts: This test aims to eliminate the extracts which at 10 mg/ml do not inhibit the growth of bacteria (Eloff, 1998). The extracts are prepared to a concentration of 20 mg/ml in a mixture acetone/Mueller Hinton broth (v/v). The obtained solutions are then homogenized using vortex. 100 µl of each extract at 20 mg/ml were distributed in triplicate wells of a 96 well plate containing previously 100 µl of bacterial broth at 10⁶ CFU/ml. The plates were homogenized using a mixer and then incubated at 37°C. After 18 h of incubation, 40 µl of a 0.2 % of *p*-iodonitrotetrazolium (INT) (Sigma Aldrich) in distilled water, were added to each well

Table 1
 yield for each extraction of *C. equisetifolia* and *O. corniculata*

Solvents	Plant material (g)	<i>C. equisetifolia</i>	<i>O. corniculata</i>
		% yield	% yield
DM	100	0,72	4,66
Me	100	8,99	1,38
HE	50	5,54	13,18

DM: dichloromethane; Me: methanol; HE: Hydroalcoholic

and the plates were incubated again at 37°C. After 30 min of incubation, the color change of the contents of the wells (extract color to red) indicates bacterial growth in wells and led to eliminate non-active extracts. The Wells which maintained their color after the addition of INT contains active extracts.

Maceration:

It is a process of letting stay solid material in a liquid to extract soluble compounds, or solid material that absorbs the liquid to get the fragrance or flavor.

Minimum Inhibitory Concentration (MIC): The Minimum Inhibitory Concentrations (MICs) were determined by the method of broth microdilution using *p*-iodonitrotetrazolium (INT) as an indicator of bacterial viability (Keymanesh et al. 2009). To determine the MIC of extracts, 100 µl of Mueller Hinton broth (DIFCO) were added to each wells of a 96-wells microplate and then 100 µl of plant extract (20 mg/ml) were added to the first well (A) of the plate. A two-fold Successive dilution was carried from well (A) to the last wells (H) of the plate. Then, 100µl of bacterial broth at 10⁶ CFU/ml were finally added into all the wells. The plate was covered and incubated at 37°C. After 18 h of incubation, 40 µl of *p*-iodonitrotétrazolium (0.2 %) was added in all wells and the plates were incubated again at 37°C. After 1 h of incubation, wells were examined and the MIC values were recorded.

Total activity: To select the extracts that can be used for further testing, the determination of the total activity is important because since the MIC value is inversely proportional to the amount of antimicrobial extracts. The total activity of each extract was calculated by dividing the MICs with the amount of extract obtained from 1 g of plant material (Eloff, 2008). This value indicates the volume in which the active principle (extract) present in 1 g of dry plant material can be diluted to always have inhibitory activity against organisms (Eloff, 2004).

2.2.3. Brine Shrimp Lethality Bioassay

The assay was performed as described by Keymanesh et al. (2009). To obtain mature naupli larva, the eggs of brine shrimp were hatched in normal seawater for 72 h. The stock solution of each extract (3 mg/ml) was obtained by dissolving 15 mg in 200 µL ethanol and 4.80 ml of seawater. Then, 1 ml of seawater containing 15 living naupli was added to 1 ml of extracts. Six concentrations ranging from 1.5 to 0.075 mg/ml, obtained by a twofold dilution of stock solution, were tested. Each experiment was done in triplicate and control was prepared using only seawater plus 15 livings naupli. Survivors naupli were counted after 24 h and dead naupli at each concentration were determined. The percentage of lethality of the brine shrimp was then calculated.

3. RESULTS

The Dichloromethane, methanol and hydroalcoholic extracts of two traditional Beninese pharmacopoeias, *Oxalis corniculata* and *Casuarina equisetifolia*, were screened for their biological properties.

3.1. Extraction yield

The yield of each extraction is mentioned in Table 1. Methanolic extraction of *C. equisetifolia* gave the highest yield (8.99%), while the best extraction efficiency of *O. corniculata* was obtained with hydroethanolic extraction (13.18%). The hydroethanolic and dichloromethane extraction of *O. corniculata* have a higher yield (13.18% and 4.66%) compared to those of *Casuarina equisetifolia* (5.54% and 0.72%).

3.2. Antibacterial activity

Growth inhibitory effect of extracts: All extracts demonstrated antibacterial activity by inhibiting the growth of one or more tested bacteria (Table 2). The hydroethanolic extract (10 mg/ml) of *O. corniculata* is the most active by inhibiting all tested bacteria. The dichloromethane extract of *O. corniculata* also inhibits the growth of six bacteria except *S. abony*. Similarly, the methanol and dichloromethane extracts of *C. equisetifolia* are also active by inhibiting five bacteria out of seven. *P. aeruginosa* is the most sensitive bacteria its growth was inhibited by all the tested extracts. *S. abony* is resistant to five extracts six. The active extracts in these trials were selected to determine their minimum inhibitory concentrations (MIC).

Minimum Inhibitory Concentration (MIC): The Minimum Inhibitory Concentrations (MICs) of extracts are recorded in Table 3. The extracts showed MIC values ranging from 0.078 to 5 mg/ml. Extracts obtained from *O. corniculata* showed interesting antibacterial activity with MIC values ranging from 0.078 mg/ml to 5 mg/ml. The hydroethanolic

CIP:

Collection Institute
 Pasteur

Table 2

Antibacterial activity of extracts at 10 mg/ml

Growth inhibition effect of extract at 10 mg/ml						
Extracts Organisms	<i>Casuarina equisetifolia</i>			<i>Oxalis corniculata</i>		
	DM	ME	H ₂ O/EtOH	DM	ME	H ₂ O/EtOH
<i>E. coli</i> (CIP 53126)	+	+	+	-	+	-
<i>S. aureus</i> (ATCC 6538)	-	-	+	-	-	-
<i>S. epidermidis</i>	-	-	+	-	-	-
<i>S.aureus</i> methicillin resistant	-	-	-	-	+	-
<i>E. faecalis</i> (ATCC 2921)	-	-	+	-	-	-
<i>P.aeruginosa</i> (CIP 82118)	-	-	-	-	-	-
<i>S. abony</i> (CIP 8039)	+	+	+	+	+	-

DM: dichloromethane; ME: methanol; H₂O/EtOH: Water/ethanol; -: inhibition; +: no inhibition**Table 3**Minimum Inhibitory Concentration of extracts from *C. equisetifolia* and *O. corniculata* extracts

Minimum Inhibitory Concentration (mg /ml)						
Extracts Organismes	<i>Casuarina equisetifolia</i>			<i>Oxalis corniculata</i>		
	DM	MeOH	H ₂ O/EtOH	DM	MeOH	H ₂ O/EtOH
<i>E. coli</i>	-	-	-	2.5	-	2.5
<i>S.aureus</i>	> 5	2.5	-	2.5	2.5	5
<i>S. epidermidis</i>	> 5	2.5	-	2.5	2.5	0.078
<i>S.aureus</i> MR	2.5	0.625	0.625	0.313	-	2.5
<i>E.faecalis</i>	> 5	2.5	-	5	2.5	2.5
<i>P. aeruginosa</i>	> 5	2.5	2.5	2.5	2.5	2.5
<i>S. abony</i>	-	-	-	-	-	2.5

DM: dichloromethane; MeOH: methanol; H₂O/EtOH: Water/ethanol; -: not active**Table 4**

Total Activity of extracts

Extracts Organismes	Total activity (ml/g)					
	<i>Casuarina equisetifolia</i>			<i>Oxalis corniculata</i>		
Extract from 1g (mg)	17.94	89.91	55.48	46.66	13.84	131.96
	DM	MeOH	H ₂ O/EtOH	DM	MeOH	H ₂ O/EtOH
<i>E. coli</i>	-	-	-	18.64	-	52.8
<i>S. aureus</i>	<1.45	36	-	18.64	5.53	26.4
<i>S. epidermidis</i>	<1.45	36	-	18.64	5.53	1689.7
<i>S. aureus</i> MR	3	144	89	149	-	52.8
<i>E. faecalis</i>	<1.45	36	-	9.3	5.53	52.8
<i>P. aeruginosa</i>	<1.45	36	22	18.64	5.53	52.8
<i>S. abony</i>	-	-	-	-	-	52.8

DM: dichloromethane; ME: methanol; H₂O/EtOH: Water/ethanol; -: not active

Table 5

Brine shrimp lethality assay of extracts from *C. equisetifolia* and *O. corniculata*

Species	Extracts	LC ₅₀	R ²
<i>Casuarina equisetifolia</i>	DM	22.02	0.96
	MeOH	8.76	0.94
	H ₂ O/EtOH	6.47	0.90
<i>Oxalis corniculata</i>	DM	13.91	0.92
	MeOH	26.87	0.98
	H ₂ O/EtOH	8.16	0.95

DM: dichloromethane; MeOH: methanol; H₂O/EtOH: water/ethanol

Artemia salina:

It is a species of crustacean lives in salt lakes, lagoons and salt marshes. It is used to evaluate the preliminary toxicity of extracts, compounds or wastewater.

SARM:

Staphylococcus aureus methicillin resistant

and dichloromethane extracts of demonstrated highest inhibition toward *S. epidermidis* and *S. aureus* Methicillin Resistant with MIC values of 78 µg/ml and 313 µg/ml respectively. The MIC values obtained with *C. equisetifolia* extracts range from 0.625 mg/ml to 5 mg/ml and the best activities were obtained with methanol and hydroalcoholic extracts with MIC value of 0.625 mg/ml against *Staphylococcus aureus* methicillin resistant (SARM). Overall, the antibacterial activity of extracts from *O. corniculata* is higher than extracts from *C. equisetifolia*.

Total activity: The total activity of active extracts has been calculated (Table 4). The extracts with higher total activity (TA) values are considered the best. The most interesting total activities of *O. corniculata* were obtained with dichloromethane (149 ml) and hydroethanol (1689.7 ml) extracts against *Staphylococcus aureus* methicillin resistant (MRSA) and *S. epidermidis* respectively while the methanol (144 ml) and hydroethanol (89 ml) extracts of *C. equisetifolia* showed best total activity against *Staphylococcus aureus* methicillin resistant with (MRSA).

3.3. Artemia salina toxicity assay

Brine shrimp lethality test results are showed in Table 5. The LC₅₀ values of the tested extracts ranged between 6.47 and 26.87 mg/ml. The highest LC₅₀ were obtained with the dichloromethane extract of *C. equisetifolia* (LC₅₀ = 22.02 mg/ml) and methanol extract of *O. corniculata* (LC₅₀ = 26.87mg/ml). The hydroethanolic and methanol extracts of *C. equisetifolia* and, hydroethanolic extract of *O. corniculata* were more toxic to shrimp with LC₅₀ of 8.76, 6.47, and 8.16 mg/ml, respectively.

4. DISCUSSION

Medicinal plants are sources of antimicrobial agents, which can be exploited in the management of human diseases. The plants are used medicinally in different countries of the world and are a good source of many potent and powerful drugs (Mahesh and Satish, 2008). Resistance of pathogens to antibiotics and antifungal commonly used, the increase in opportunistic infections and the effect of toxicity due to the continued use of several drugs have led to increased attention paid to the search for new therapeutic agents from various sources, including plants, which are good starting materials for the discovery of new antimicrobial agents (Sasidharan et al. 2011; Saad et al. 2011). In the present study, biological activities of two medicinal plants of Benin pharmacopeia have been investigated.

The MIC values obtained with *O. corniculata* extracts range from 0.078 mg/ml to 5 mg/ml and the hydroethanolic extract was the most active with MIC values of 0.078 mg/ml and 5 mg/ml against *S. epidermidis* and *S. aureus* respectively. Similar results have been obtained with aqueous extract of *O. corniculata* (CMI = 6 mg/ml) against *S. aureus* (Satish et al. 2008). MICs obtained with *C. equisetifolia* extracts range from 0.625 mg/ml to 5 mg/ml and methanol and hydroethanolic extracts showed the best activities with MIC value of 0.625 mg/ml against *Staphylococcus aureus* methicillin resistant (SARM). Our results are more interesting than those obtained by Nehad and Abdulrahman (2012) in which the methanol extract showed a MIC of 50 mg/ml against the same bacteria. It is highly probable that the antibacterial compounds of fruits of *C. equisetifolia* and leaves of *O. corniculata* are more soluble in the hydroalcoholic solvent. This explains the strong antibacterial activity of hydroethanolic extract against Gram positive and Gram negative bacterial strains. Usually, the use of alcohol as extractant is often encouraged to extract antimicrobial compounds from plant materials (Selowa et al. 2010; Sati and Joshi, 2011; Olajuyigbe and Afolayan, 2012).

The methanol and hydroethanol extracts gave a MIC value of 62.5 µg/ml against *Staphylococcus aureus* methicillin resistant. This is in agreement with work by Ahsan et al. (2009), in which they obtained a MIC value of 64 µg/ml for methanol extract of *C. equisetifolia* against *S. aureus*. These comparable results suggest that polar extracts of *C. equisetifolia* are more active against of *S. aureus* strains. Brine shrimp lethality assay showed interesting activity with LC50 values ranging from 6.47 to 26.87 mg/ml. These interesting results indicate that none of the extracts are toxic to larvae. This is confirmed by the work of Zakaria et al. (2007) in which the author states that extracts are toxic when the LC₅₀ is less than 100 µg/ml. Thus, we concluded that all extracts tested in this study exhibited very low or no toxicity, giving LC₅₀ values higher than 100 µg/ml.

5. CONCLUSION

This study confirmed the traditional use of *O. corniculata* and *C. equisetifolia* and suggests that some of the extracts having antibacterial properties can be further explored as a possible antibacterial agent source for the management of infectious pathogenic diseases. The most important result was that hydroethanolic extract of *O. corniculata* revealed significant antibacterial effect against *S. epidermidis*.

SUMMARY OF RESEARCH

This work was used to evaluate the antibacterial and preliminary toxicity of two medicinal plants. The findings give a scientific basis to the traditional uses of *O. corniculata* and *C. equisetifolia*.

FUTURE ISSUES

It is still unknown which compounds are responsible for the biological activity of the two medicinal plants. Thus, bioassay-guided isolation and identification of the active secondary metabolites of these plants will be process.

ACKNOWLEDGE

The authors are grateful to the medicinal plants seller and traditional practitioners from Ouémé and Ouidah regions. Helpful work of Botanist, Dr. Yedomohan, from Herbarium National of University of Abomey-calavi is appreciated.

DISCLOSURE STATEMENT

There is no financial support for this research work from the funding agency.

REFERENCE

1. Doughart JH, Okafor B. Anti-microbial activity of *Senna alata* Linn. East and Central Afr J. Pharm. Sci., 2007, 10, 17-21
2. George WB. A review of pathogenic microbiology Saint Louis. CV Mosby Company. 1974
3. Soforowa A. Medicinal plants and traditional Medicine in Africa. 1st ed. Spectrum Books Ltd., Ibadan, Nigeria, 1982
4. Ngo LT, Okogunb JL, Folk WR. 21st Century natural product research and drug development and traditional medicines. Nat. Prod. Rep., 2013, 30, 584-592
5. Kennedy DO, Wightman EL. Herbal extracts and phytochemicals: Plant secondary metabolites and the enhancement of human brain function. Adv. Nutr., 2011, 2, 32-50
6. Adedapo AA, Jimoh FO, Afolayan AJ, Masika PJ. Antioxidant properties of the methanol extracts of the leaves and stems of *Celtis Africana*. Records of Natural Products, 2009, 3, 23-31
7. Bolou GEK, Attioua B, N'Guessan AC, Coulibaly A, N'Guessan JD, Djaman AJ. Evaluation in vitro de l'activité antibactérienne des extraits de *Terminalia glaucescens* planch sur *Salmonella typhi* et *Salmonella typhimurium*. Bulletin de la Société Royale des Sciences de Liège, 2011, 80, 772-790
8. Traoré Y, Ouattara K, Yéo D, Doumbia I, Coulibaly A. Recherche des activités antifongique et antibactérienne des feuilles d'*Annona senegalensis* Pers. (Annonaceae). Journal of Applied Biosciences, 2012, 58, 4234-4242
9. Eloff JN. A sensitive and quick method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med., 1998, 64, 711-713
10. Keymanesh K, Hamed J, Moradi S, Mohammadipناه F, Sardari S. Antibacterial, antifongique and toxicity of rare Iranian plants. Int. J. Pharm., 2009, 5, 81-85
11. Eloff JN, Katerere DR, McGaw LJ. The biological activity and chemistry of the southern African Combretaceae. J. Ethnopharmacol., 2008, 119, 686-699
12. Eloff JN. Quantification the bioactivity of plant extracts during screening and bioassay guided fractionation. Phytomedicine, 2004, 11, 370-371
13. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plants against plant and human pathogens. World J. Agric. Sci., 2008, 4, 839-843
14. Sasidharan S, Prema B, Yoga LL. Antimicrobial drug resistance of *Staphylococcus aureus* in dairy products. Asian Pac. J. Trop. Biomed., 2011, 1, 130-132
15. Saad S, Taher M, Susanti D, Qaralleh H, Rahim NABA. Antimicrobial activity of mangrove plant (*Lumnitzera littorea*). Asian Pacific journal of tropical medicine, 2011, 4, 523-525
16. Satish S, Raghavendra MP, Raveesha KA. Evaluation of the Antibacterial Potential of some plants against human pathogenic bacteria. Adv. Biol. Res., 2008, 2, 44-48
17. Nehad MG, Abdulrahman SH. Antimicrobial efficacy of *Casuarina equisetifolia* extracts against some pathogenic microorganisms. J. Med. Plants Res., 2012, 6, 5819-5825
18. Selowa SC, Shai LJ, Masoko P, Mokgotho MP, Magano SR. Antibacterial activity of extracts of three *Croton* species collected in Mpumalanga region in South Africa. Afr. J. Trad. CAM, 2010, 7: 98-103
19. Sati SC, Joshi S. Aspects of antifungal potential of ethnobotanically known medicinal plants. Res. J. Med. Plant, 2011, 5, 377-391
20. Olajuyigbe OO, Afolayan AJ. In vitro pharmacological activity of the crude acetone extract of *Erythrina caffra* Thunb: antibacterial and antifungal assessment. Journal of Medicinal Plants Research, 2012, 6, 1713-1720
21. Ahsan R, Islam M, Haque E, Mossaddik A. In vitro antibacterial screening and toxicity study of some different medicinal plants. World Journal of Agriculture Sciences, 2009, 5, 617-621.
22. Zakaria HM, Mainen JM, Pax JM, Modest CK, Ramadhani SON. Antimicrobial activity and brine shrimp toxicity of extracts of *Terminalia brownii* roots and stem. BMC Complementary and Alternatives Medicine, 2007, 7, 1-5