PHARMACEUTICAL SCIENCES

RESEARCH ARTICLE

CHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF EXTRACTS FROM THE FLOWERS AND STEM OF *HYPHAENE THEBAICA* L.(ARECACEAE)

B. SEID ABAKAR[1], P.R. FOTSING KWETCHE[2,3]*, J. NGOUPAYO[1], T. TABOPDA KUATE[4], J. KUOAMOUO[2,3], A.B. BECHIR[5], M.A. MAFO FOKAM[2,3] A.M. CHAHAD[6], A.B. MAHAMAT[7], and E. PONDO PONDO[1],

[1] Faculty of Medicine and Biomedical Sciences; Yaoundé-Cameroon
[2] Université des Montagnes; Bangangté-Cameroon
[3] Cliniques Universitaires des Montagnes; Bangangté-Cameroon
[4] Faculty of Science, University of Yaoundé I; Yaoundé-Cameroon
[5] Université des Sciences et Technologie d'Ati; Ati- Chad
[6] Institut Universitaire des Sciences et Techniques d'Abéché; Abéché-Chad
[7] University of Ngaoundéré; Ngaoundéré-Cameroon

*Corresponding author e-mail: prfotsing@udesmontagnes.org

Abstract

The present investigation addressed a semi-quantitative chemical screening of a few known bioactive secondary metabolites and the antimicrobial potentials of crude extracts from the flowers and stem of *Hyphaene thebaica* (*H. thebaica)*. Those chemicals targeted included: alkaloids, coumarins, flavonoids, anthocyanins, mucilages, glycosides, polyphenols, quinones, saponosids and terpenoids. The solvents used were methanol and ethyl acetate. The antimicrobial activity was tested according to standard protocols on nine bacterial strains from both Gram types and *C. albicans*. This was done through determination of the minimal inhibitory and minimal lethal concentrations; and the diameters of growth inhibition. Almost all chemicals targeted were present in both crude extracts but extraction with methanol was more effective than extraction with ethyl acetate. At varying levels regardless of the Gram type and the origin of the extracts, the antimicrobial activity extended to all tested organisms. The MIC values ranged from 0.1 through 50 mg/mL. This activity covering all Gram types of bacteria and *C. albicans* corroborate the use made of *H. thebaica* by indigenous health practitioners, consistent with the WHO advocacy about the clinical significance of improved traditional drugs this plant appears as a reliable candidate.

Keywords: Chemical screening, *Hyphaene thebaica*, antimicrobial activity

Introduction

Throughout centuries and generations, humans have empirically used the virtues of natural resources to tackle their daily issues including the struggle against physiological and infectious diseases (IDs) (Dahiru, 2013). With the advent of modern microbiology in the 1865s, the challenge against IDs became much more oriented and opened ways to development of regimens of antimicrobial agents that obviously reduced the rates of deaths and increased life expectancy in many populations across the globe. The discovery of resistance phenotype in micro-organisms noticed soon after re-tabled, however, the issues of IDs that remain a permanent challenge in all health systems, associated with the interplay between drug use and

microbial drug tolerance (Iserin *et al.,* 2001; Fotsing Kwetche *et al.,* 2012; Tamil Selvan, 2013).

Some developed nations can afford the most recent conventional drugs, health facilities and healthcare workers why others, basically in developing countries do not have access to the minimum they need (Andersson and Hughes, 2011; Fotsing Kwetche *et al.,* 2012; Mondal *et al.,* 2012; Anochie *et al.,* 2013). Further, antimicrobial resistance is a global phenomenon. Its fast growth is alarming among populations with limited resources in developing countries (Andersson and Hughes, 2011; Nguendo Yongsi, 2011; Tamil Selvan, 2013). Microbial resistance is associated with social determinants like basic education, average living standard, technological advances, and human resources for health. These factors are directly linked with inadequate use of available modern drugs in human medicine, veterinary medicine and crop production. Beyond the fast resistance growth, it is estimated that 10-20% of hospital admissions are due to the side effects of conventional drugs. Moving back to original but modernized and scientifically supported practices like the use of resources from plants (versatile resource worldwide) appeared as a reliable option for many. According to some reports (Iserin *et al.*, 2001; WHO, 2003) in addition, some traditional drugs are more effective than conventional ones and can be used in resource limited settings. Estimating that 50% of all deaths in tropical countries are caused by infectious diseases and that more than 80% of people in Africa rely on traditional medicine, the WHO encouraged the production of improved traditional drugs (ITDs) that would be available and cost effective in specific contexts. In general, plants are rich in large varieties of bioactive components like essential oils, alkaloids, terpenoids, flavonoids, tannins, coumarins, phenolic compounds and heterosides acknowledged as potent substances against infectious agents. Many attempts are underway in understanding the scientific bases of antimicrobial phytotherapy and identifying associated bioactive components with their respective roles in the overall effectiveness of crude extracts (Igbinosa *et al.*, 2009; Chougouo kengne *et al.,* 2012; Kouitcheu Mabeku *et al.,* 2013). A wide range of secondary metabolites are currently known in many plants with varying concentrations, depending upon the climate under which they are grown, the plant part and the soil composition (Chouguouo Kengne *et al.,* 2012; Kuate Tokam *et al.,* 2013).

The present study targeted the phytochemical screening and antimicrobial potentials of extracts from *Hyphaene thebaica* (*H. thebaica*) harvested in Haraza (Republic of Chad - Central Africa). This work was conducted in the framework of the ongoing research program on medicinal plants put in place by health institutions in many African countries. It aimed at determining through macro-dilution the minimal inhibitory concentrations (MICs), the minimal lethal concentrations (MLC-minimal bactericidal and fungicidal concentrations-) of extracts from the flowers and stem of this plant. The inhibitory zone diameters associated with the MICs and MBCs were also assessed for a few strains with and without DMSO. Antimicrobial activity was tested on a total of nine bacterial and one dimorphic fungal strains belonging to microbial species often incriminated in IDs (either as true pathogens or as opportunistic organisms). The goal was to broaden the field of investigations in order to contribute to the development of new IDs treatment alternatives with potential sources for new conventional drugs.

Material and Methods

a. Plant identification and work sites

The plant identification was conducted by botanists in Chad and confirmed at the National Herbarium in Cameroon under the collection number 25255/SRF$_{Cam}$. The 8-10 years-old plant pats were harvested in October, 2013. Thereafter, the phytochemical screening was conducted in the Laboratories of chemistry of the Faculty of Medicine and Biomedical Sciences, Higher Teacher's Training College, Yaoundé-Cameroon, and the antimicrobial tests performed in the Laboratory of Microbiology (Université des Montagnes, Bangangté-Cameroon).

Extractions were carried out on dried flowers and stem with methanol and ethyl acetate. 7 Kg and 5 Kg of the powdered product (flower and stem, respectively) were macerated in 15 L of each of the two solvents for 72 h. Filtration with whatman paper No 1 followed. The filtrates obtained were further concentrated in a Rota-Vapor (HEIDOLPH, HEIZBAB®) at 45°C and 200 bars to generate the crude residues used in the chemical screening.

b. Phytochemical screening

The aqueous test solution was prepared by dissolving (stirring to homogenization with a

magnetic agitator) 1 g of each crude extract in 20 mL of distilled water.

1. Test for Alkaloids (Mayer's, Hager's and Wagner's tests)

2 mL of the test solutions were transferred into three test tubes. 2 mL of distilled water were also put in three other test tubes (negative controls). Respectively, a few drops of Mayer's, Hager's and Wagner's reagents were added in the first group of tubes that contained the extracts, and in the three negative control tubes. The presence of alkaloids was evidenced by the development of precipitates in the 3 tubes that contained the test solutions, unlike the 3 control tubes (Djeussi *et al.,* 2013).

2. Test for Coumarins (Alkaline reagent test)

In a test tube, 2 mL of NaOH was added to 2 mL of the test solution in each case. Development of a greenish yellow or blue fluorescence indicated a positive test for coumarins (Alzoreky and Nakahara, 2003).

2. Test for flavonoids

General tests

When a few drops of $FeCl_3$ were added to 2 mL of the extract in a test tube, development of an intense green color was evidence of the presence of flavonoids.

To 2 mL of the test solution 3 mL of NaOH was added. If a yellow color developed and discolored upon addition of HCl, that indicated the presence of flavonoids in the extract (Alzoreky and Nakahara, 2003).

Specific Test (Anthocyanins)

To 2 mL of each crude sample, 2 mL of H_2SO_4 was added. A pink-red color that turned into purple blue with addition of ammonia indicated the presence of anthocyanins (Ayoola *et al.,* 2008).

3. Test for glycosides

About 1 mL of chloroform and 1 mL of 10% ammonia were respectively added to 2 mL of the extracts in the test tubes. A pink-red color indicated a positive test.

4. Mucilages

Two tubes were used in each case. 2 mL of each extract were transferred in the first and 2 mL of distilled water in the second. In each of these tubes, 3 mL of 95% ethanol were added. A flake formation confirmed the presence of mucilages (Sofowora, 1993).

5. Polyphenols

Two tubes were used in this experiment: the test and the control. 2 mL of each extract were transferred into test tubes and 2 mL of distilled water in the control tubes. Development of a whitish precipitate upon addition of lead acetate indicated the presence of total phenols. - When 2 mL of the test solution were mixed with 1 mL of acetic acid, development of a reddish color indicated the presence of polyphenols.

6. Saponosids test (frothing test)

2 mL of each test solution were introduced in a test tube containing 2 mL of distilled water. The tube was stopped and shaken vigorously for about 15 seconds. Allowed to stand for 15 min, persistent frothing indicated the presence of saponosids (Sofowora, 1993).

7. Test for terpenoids (Chloroform test)

2 mL of chloroform were mixed with 2 mL of the test solutions. To this mixture, 2 mL of concentrated H_2SO_4 were added and heated for 120 s in a water bath (65°C). A reddish brown color that developed at the interface was evidence of the presence of terpenoids **(**Mahesh and Satish, 2008)**.**

8. Test for quinones

When 2 mL of the test solution were mixed with 2 mL of concentrated H_2SO_4, color changing into red was evidence of the presence of quinones. In each of the above cases, positive tests were semi-quantified based on the intensity of the test indicator.

c. *In vitro* antimicrobial susceptibility assays
Test micro-organisms

The micro-organisms used in this investigation were selected on the basis of their clinical and pharmacological importance. They included six Gram-negative bacterial strains (*E. coli* ATCC 25922, *E. coli* ATCC 35218, *Pseudomonas aeruginosa* QC 76110*, Salmonella* Enteritidis*, Serratia odorifera* and *Vibrio cholera* O1); two Gram-positive cocci (*S. aureus* QC 1625 and *S.*

94

aureus ATCC 1026[TM]); one Gram-positive bacillus (*Enterococcus faecalis* ATCC 29212), and a dimorphic fungal specie, *Candida albicans.*

Minimal Inhibitory Concentration (MIC)

Prior to the MIC tests, all microbial strains were seeded on Mueller Hinton agar (Liofilchem) and incubated at 37°C for 24 h aerobically. From the resulting overnight pure culture, a bacterial suspension equal to 0.5 McFarland (10^6-10^8 cells/mL) was prepared and adjusted to the final density required for susceptibility tests according to the ''Comité de l'Antibiogramme de la Société Française de Microbiologie, CA-SFM (2014)''. A similar procedure was followed to prepare, from a fresh culture on Sabouraud agar (Liofilchem), the 2×10^6 cells/mL suspension density used to assess the antifungal activity with conventional techniques. The test was conducted by macro-dilution in Mueller Hinton broth (MHB) as performed in Kuate Tokam *et al.,* (2013) with slight modifications. Briefly, the original (stock) solution was prepared at 400 mg/mL. In each of a series of twelve test tubes, 2 mL of MHB was dispensed. From this preparation, a serial dilution was performed (from the stock solution) to obtain extract concentrations that ranged from 200 mg/mL through 0.1 mg/mL. In addition, two controls were prepared with MHB without extract (positive control for growth and the negative control for broth sterility). This procedure was repeated for all microbial strains (*C. albicans* was tested in Sabouraud broth).

In each tube (except the broth sterility control), 15 µL of the above bacterial (or fungal) preparation were added. The preparations were incubated at 37°C for 24 h for bacteria and 30°C for *C. albicans* in aerobic environments. When incubation was completed, the tubes were centrifuged at 5000 rpm for five minutes. The MICs were read from the first tube in which no microbial precipitate (whitish) was observed at the bottom upon centrifugation (absence of growth), and confirmed by the broth turbidity recorded prior to centrifugation. This procedure was repeated three times for each strain for test reproducibility.

Minimal Lethal Concentration (MLC)

The tubes in which no visible microbial growth was obtained were re-homogenized. 10-15 µL of the resulting suspension were reseeded on appropriate agar (Mueller Hinton agar for bacteria and Sabouraud agar for *C. albcans*). These cultures were allowed to incubate aerobically overnight at 37°C and 30°C, respectively. Upon completion of incubation the MLC (MBCs for bacteria and MFC for *C. albicans*) were recorded from the first dilution in which no visible growth was obtained on the agar plate.

Microbial growth inhibition zone diameters

The inhibitory zone diameters were determined by the disc diffusion technique on MHA (Liofilchem) according to Hayes and Markovic (2002) with slight modifications. Briefly, the bacterial suspension prepared as described above was further diluted (1/10) to the final density required for susceptibility test by standard agar diffusion technique with swabs.

On the seeded agar plate, ten sterile paper discs (6 mm diameter each) were deposited for the tests. Out of these ten paper discs, 15 µL of the extract at the MIC concentration were dispensed on four (one received sterile distilled water) on one hand; 15 µL of the extract at the MIC concentration with 10% (v/v) DMSO (Merk) was dispensed on four and one was inoculated with DMSO. The same procedure was followed at the MBCs for all bacterial strains tested. These preparations were, thereafter, incubated aerobically at 37°C for 18-24 h. After incubation, the zone of inhibition around each disc was recorded and computerized in terms of means and standard deviations.

Results

Phytochemical screening of the crude extracts

The solvents used displayed various effectiveness in the extraction process. The results obtained in this semi-quantitative assay were recorded, summarized and presented in table 1.

When present in the plant, the bioactive compounds targeted were indiscriminately detected in approximately similar amounts in the flowers and stem. It also appeared from the tabled data that extraction with methanol was more effective than with ethyl acetate in either part. Overall in fact, about 80% and 70% of the chemical categories were identified in the flowers and stem, respectively, when methanol was used for extraction, against 50% and 60% with ethyl acetate. Coumarins and mucilages could not be detected from any of the crude extracts, regardless of the technique used. Acetic acid was also efficient in characterizing polyphenols only in the methanol extract from the flowers.

Table 1: Characterization of chemicals in the methanol and ethyl acetate crude extracts

Chemical category	Reagent	Flowers		Stem	
		$METH_f$	$ETAC_f$	$METH_f$	$ETAC_f$
Alkaloids	Valser-Mayer	+++	-	++	+++
	Hager	+++	-	++	+++
	Wagner	+++	+	+++	+++
Coumarins	NaOH	-	-	-	-
Flavonoids	$FeCl_3$ 1%	+++	+++	++	+
	NaOH +HCl	+++	++	++	+++
Anthocyanin (spF)	$H_2SO_4+NH_4OH$	+++	-	+++	-
Glycoside	10% Cloroform + NH_4	++	-	-	-
Mucilage	Ethanol 95%	-	-	-	-
Polyphenols	Acetate lead	+++	+	+++	+++
	Acetic acid	+++	-	-	-
Quinones	H_2SO_4	+++	-	+++	+++
Saponosids	Nr	+++	++	+++	++
Terpenoids	Chloroform + H_2SO_4	+++	+	+++	+++

$METH_f$: Extract with Methanol; $ETAC_f$: Extacts with Ethyl acetate; spF: specific flavoinoids; Nr: no particular reagent

In vitro biological activity

Assessment of the antimicrobial potentials of the crude extracts generated series of data that were computerized and summarized accordingly.

The MICs, MLCs and the inhibitory zone diameters at the MICs and MLCs recorded are displayed in table 2.

Table 2: MICs, MLCs and MLC/MICs values of the extract of flowers (upper line) and stem (lower line)

Micro-organism	MIC(mg/mL)	MLC(mg/mL)	MLC/MIC
E. coli ATCC 25922	6.25	50	8
	25	>200	8
E. coli ATCC 35218	3.13	25	8
	3.13	100	32
S. aureus QC 1625	3.13	>200	>64
	6.25	>200	>32
P. aeruginosa QC 76110	6.25	12.5	2
	50	200	4
E. fecalis ATCC 29212	3.13	50	16
	25	>200	>8
S. aureus ATCC 1026^{TM}	25	50	2
	3.13	100	32
V. cholerae O1	50	50	1
	50	200	4
S. Enteritidis	3.13	25	8
	25	>200	>8
S. odorifera	0.1	>200	>2000
	0.1	>200	>2000
C. albicans	3.13	100	32
	3.13	100	32

*   > : Larger than ; *** :-** First line: flower-derived; - second line: stem-derived

With the exception of *V. cholerae* in which they were equal, the overall picture is that the MLCs were very large compared to the MICs. The largest MIC indexes were obtained with *S. odorifera* and *S. aureus* QC 1625 in which no extract concentration within the range considered (regardless of the origin) could have a lethal effect. No lethal effect could also be obtained on *E. fecalis* ATCC 29212 and *S.* Enteritidis with the extract from the stem. On *E. coli* ATCC35218, O1-*V. cholerae*, *S. odorifera* and *C. albicans*, growth inhibition was recorded at similar concentration for both crude extracts.

When bacterial growth inhibition was assessed on MH agar the inhibitory zone diameters generated by the diffusion of extracts from the flower and stem (with and without addition of DMSO) were recorded and displayed as presented in Table 3. As expected, the diameters at the MBCs were larger than those recorded at the MICs in all cases. They differed slightly with the extract used on the same bacterial strain. But overall, addition of DMSO was followed by an increase in the zone of inhibition in most cases. The largest increase was observed with *E. coli* ATTCC 35218. In *V. cholerae*, its addition in the flower-derived extract did not produce any obvious additional effect.

Table 3: Inhibitory zone diameters with and without DMSO

Microorganism	Mean Inhibition zone diameter ± SD (mm)*			
	Without DMSO		With DMSO	
	MIC	MBC	MIC	MBC
E. coli ATCC 25922	8±1.4	9±0.0	10±0.8	10±0.8
	08±1.4	9±0.	10±0.8	10±1.4
E. coli ATCC 35218	10.3±0.9	10.5±0.5	11±0.0	11.25±0.9
	7.5±0.5	11±0.0	11±0.8	14±1.4
S. aureus ATCC 1026[TM]	9±0.8	10±0.0	11±0.8	11.5±0.9
	8±0.8	12±0.0	10±1.5	13±1.4
P. aeruginosa QC 76110	9±0.0	11±0.8	11.5±0.5	15±0.8
	8±0.8	12±0.0	10±1.5	13±1.4
V. cholerae 01	9±0.0	10±0.8	9±0.0	10±0.8
	10±0.0	10±0.8	10±1.1	13±0.8

* - upper line: flower-derived; - lower line: stem-derived

Discussion

The present investigation provided substantial pieces of information that corroborate the use of *Hyphaene thebaica* (*H. thebaica*) by traditional health practitioners. *H. thebaica* is rich in hosts of secondary metabolites that were previously identified in other plant resources (Mahesh and Satish*,* 2008; Djeussi *et al.,* 2013; Kuate Tokam *et al.,* 2013) and known for their role in protecting against and preventing human diseases. Overall, 80% of the targeted bioactive compounds were detected in the plant regardless of the part from which the crude extract was obtained, the solvent or the screening technique used. However, optimal use of the plant potential appeared to rely on methanol for extraction. This is consistent with the findings of the earlier authors who reported that appropriate choice of the solvent is important in optimizing the extraction product composition (Wendakoon *et al.,* 2012). The bioactive phyto-compounds detected (and semi-quantified) in *H. thebaica* roughly included alkaloids, flavonoids, glycosides, terpenoids, saponosids, polyphenols and quinones. Flavonoids and alkaloids were described as antibacterial, antiviral and anticancer agents (Ayoola *et al.,* 2006; Ayoola *et al.,* 2008; Savithramma *et al.,* 2011), among other potentials they possess. Extracts from *H. thebaica* could also play a key role in the control of haemostasis and the related vasoconstriction with regard to the presence of saponins and terpenoids (Dandjesso *et al.*, 2012). Saponosids can act and reduce blood cholesterol, blood sugar or cause weight loss. Further, they can be used as detergent, pesticide, molluscicide, anifungal, or as anticancer agents (Rathore *et al.,* 2012).

 In general, plant extract may act on microorganism through different mechanisms, some of which include complexation of the cell envelop constituents (and the associated disruption of the normal cell metabolic processes), and inhibition of the quorum sensing mechanism (Khan *et al.,* 2009; Szabo *et al.,* 2010). Both have paramount importance in the infectious process (microbes require convenient inoculums to cause disease in their hosts). Although the individual role of each

constituent is yet to be fully investigated through, it is known that flavonoids attach to extra-cellular and membrane-borne proteins, cause their inactivation while quinones interfere with bacterial survival by inactivation through attachment to nucleophilic amino acids within protein molecules. It is not clear, however, whether *in vivo* the bioactive compounds play neutral, additional, synergetic or antagonistic roles. A quantitative study is necessary to determine both the content and activity of each of the chemicals detected, and perhaps, would be useful in understanding better why one extract is more effective than the other. *P. aeruginosa*, *S. odirifera* and *E. coli* are representative of a large group of Gram-negative bacteria that are commonly incriminated in deadly opportunistic nosocomial and community acquired infections. Their role in the infectious process can be regarded in at least two ways: their virulence that is associated with rapid growth (15-20 min/generation) and the resistance traits they may express or disseminate through genetic mobile elements. Both factors are likely to prolong the course of infection with the related extra financial charges (Yorita-Christensen *et al.,* 2009). Professional Gram-negative bacilli like *V. cholerae* or *S.* Enteritidis are occasionally incriminated (because they are not as frequent as the formers in nature). They can cause outbreaks or severe food-borne infections in areas with low hygiene standard (Tamatcho Kweyang *et al.,* 2012; Kouitcheu Mabeku *et al.,* 2013). In the present study the strains tested were susceptible at relatively low extract concentration.

Strains of *S. aureus* are incriminated in suppurative infections, superficial lesions (like boils and impetigo), throat sore, pneumonia, mastistis, phlebitis, meningitis, food poisoning, and urinary tract infections (Wendakon *et al.,* 2012; Sen and Amla, 2012; Daniel *et al.,* 2012; Djeussi *et al.,* 2013). Strains used in the present investigation were also found susceptible at relatively low concentrations.

These antimicrobial tests revealed very slight differences in the activity of both extracts, consistent with result obtained in the chemical screening. The differences may be suggesting that other active constituents (beyond the identified ones) were likely present in the extract. Activity recorded in the present investigation is evidence that the crude extracts could be used in therapeutic, in accordance with the use made if the plant by traditional practitioners in human IDs management. This assertion can be reinforced by the relatively reduced values of MICs recorded (the highest MIC observed was 50 mg/mL).

Earlier surveys (Aboada and Efuwape, 2001; Mohanta *et al.,* 2007; Sen and Amla*,* 2012) reported that saponins could inhibit growth in *C. albicans***.** Accordingly, growth inhibition of *C. albicans* (at reduced MIC) could be explained by the high saponin amount in the crude extract. Related conclusions could also be drawn as far as other active secondary metabolites are concerned. From these findings, however, it cannot be ruled out that one component or the other was actually responsible for the antimicrobial effect observed (for the reasons stated above). Wendakoon *et al.* (2012) reported differential effectiveness of plant extract on Gram-positive and Gram-negative bacteria. In the current investigation, the action appeared to be strain-specific rather than Gram-group specific and, like in Kuate Tokam *et al.* (2013), addition of DMSO further potentialized the activity of the extracts.

The use of extracts from this plant, therefore, appears as a reliable alternative that can be capitalized in resource-limited communities were conventional drugs are either lacking or, available but beyond the purchasing power of most people. Further concentrating the extract would produce even more satisfactory results. This requires proper orientation of the local heath policy and will be possible through standardized extraction techniques and solvents that should be specified according to the targeted bioactive compounds (once detected) in the natural resources of interest. One of the paramount virtues of plant extracts is that they contain a host of chemical compounds that have potentials to act and protect against many human conditions, unlike modern drugs and food additives, generally more focus.

Conclusion

Hyphaene thebaica provides a precious available and sustainable source for medicine in the short run for indigenous populations and a reliable source for new conventional drugs. This promising future with *H. thebaica* and other natural resources relies on a strong political will, financial support, trained and motivated human resources that will explore the diversity of their potentials for human welfare.

Acknowledgments

References

Aboada, O.O. and Efuwape, B.M. 2001. Antibacterial properties of some Nigerian species. *Biol. Res. Comm.* 13: 183-188.

Alzoreky, N.S. and Nakahara, K. 2003. Antibacterial activity of extracts of some edible plants commonly consumed in Asia. *Int. J. Food Microbiol.* 80:223-230.

Andersson, D.I. and Hughes, D. 2011. Persistence of antibiotic resistance in bacterial populations. *FEMS Microbiol. Rev.* 35: 901-911.

Anochie, P.I., E.C. Onyeneke, E.O. Asowata, E. Afocha, A.C. Onyeozirila, A.C. Ogu and Onyeneke, B.C. 2013. The role of rotavirus associated with pediatric gastroenteritis in a general hospital in Lagos, Nigeria. *Imerg. Infect. Dis.* 3:81-89.

Ayoola, G.A., T. Sofidiya, O. Odukoya and Coker, H.A.B. 2006. Phytochemical screening and free radical scavenging activity of some Nigerian medicinal plants. *J. Pharm. Sci. Pharm. Pract.* 8: 133-136.

Ayoola, G.A., H.A.B. Coker, S.A. Adesegun, A.A. Adepoju-Bello, K. Obaweya, E.C. Ezennia and Atangbayila, T.O. 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Trop. J. Pharm. Res.* 7: 1019-1024.

Chougouo Kengne, R.D., P.R. Fotsing Kwetche, J. Kouamouo, B. Domum Tchouanche, R. Somo Moyo and Kaptué, L. 2012. Antibacterial and antifungal activity of the essential oil extracted by hydro-distillation from *Artemesia Annua* grown in West-Cameroon. *Br. J. Pharmacol. Toxicol.* 4: 89-94.

Dahiru, D. 2013. Protective effect of Bi-herbal Formulation of *Ocimum gratissimum* and *Gongronema latifolium* aqueous leaf extracts on acetaminophen-induced hephrotoxicyty in rats. *Am. J. Biochem.* 3: 18-23.

Dandjesso, C., T.V. Klotoa, J. Segbo, J-M. Ategbo, F. Gbaguidi, L. Fah, B. Fanou, F. Loko and Dramane, K. 2012. Phytochemistry and hemostatic properties of some medicinal plants sold as anti-hemorrhagic in Cotonou markets (Benin). *Indian J. Sci. Technol.* 5: 3105-3109

Daniel, U.N., C.N. Ohalete and Nnoli, M.C. 2012. Phytochemical screening for active compounds in leaves, bark and seed extracts of *Azadirachta indica* in Owerri, Imo State. South-Eastern Nigeria. *World J. Pharm. Sci.* 1: 1181-1188.

Djeussi, D.E., J.A.K. Noumedem, J.A. Seukep, A.G. Fankam, I.K. Voukeng, S.B. Tankeo, A.H.L. Kuete and Kuete, V. 2013 Antibacterial activity of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. *BMC Complmement Altern. Med.* 13:164.

Fotsing Kwetche, P.R., J. Kouamouo, P. Mbaya, D. Yangoué, Nsangou Adamou, J.P. Wona, J. Simo Louokdom, Kourouma Kaba and Kaptué, L. 2012. Antibiotic resistance in *Escherichia coli* isolated from women genitalia and trend of minimal inhibiting concentration in a semi-urban population: Bangangté, West-Cameroon. *Curr. Res. J. Biol. Sci.* 4: 696-701.

Hayes, A.J. and Markovic, B. 2002. Toxicity of Australian essential oil *Backhoussia citriodora* (Lemon myrtle). Part 1. Antimicrobial activity and *in vitro* cytotoxicity. *Food Chem. Toxicol.* 40: 535-543

Igbinosa, O.O., E.O. Igbinosa and Aiyegoro, O.A. 2009. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *Afr. J. Pharm. Pharmacol.* 3: 058-062.

Iserin, P., M. Masson, and Roslellini, J.P. 2001. Encyclopédie des plantes médicinales, *2ième Ed.* Larouuse Paris France p235.

Khan, M.S., M. Zahin, S. Hasan, F.M. Husain and Ahmad, I. 2009. Inhibition of quorum sensing regulated bacterial functions by plant essential oils with special reference to clove oil. *Lett. Appl. Microbiol.* 49: 354-360.

Kouitcheu Mabeku, L.B., E. Talla, J. Kouam, T. Zra and Oyono Essame, J.L. 2013 : Synergetic effects of plant extracts and antibiotics on *Vibrio cholerae* O1 strains isolated from clinical specimens. *I.J.B.* 5: 64-72.

Kuate Tokam, C.R., P.R. Fotsing Kwetche, J. Ngoupayo, J. Ndelo, J. Kouamouo, S. Gamwo Dongmo, G.M. Tcheuffa Ngassa, K. Younang Tonme, and Kaptué, L. 2013. Phytochemical screening, antibacterial and antifungal activity of crude aqueous and methanol extracts of stem bark of *Garcinia brevipedicellata*. *Int. J. Pharm. Biomed. Res.* 4: 221-226.

Mahesh, B. and Satish, S. 2008. Antimicrobial activity of some important medicinal plant against human pathogens. *World J. Agric. Sci.* 4: 839-843

Mohanta, K.N., S.N. Mohanty, J. Jena and Sahu, N.P. 2007. Effect of different oil cake sources on growth, nutrient retention and digestibility, muscle nucleic acid content, gut enzyme activities and whole-body composition in silver

barb, *Puntius gonionotus* fingerlings. *Aquacult. Res*. 38: 1702-1713.

Mondal, D., J. Minak, A. Masud, L. Yue, J. Dai, and Poonum, K. 2012. Contribution of enteric infection, altered intestinal barrier function, and maternal malnutrition to infant malnutrition in Bangladesh. *Clin. Infect. Dis.* 54:185-192.

Nguendo Yongsi H.B. 2011. Access and management of drinking water in developing cities: Evidence from Yaoundé (Cameroon). *Res. J. Environ. Sci.* 5:124-133.

Rathore, S.K., D.R. Shashank Bhatt, D. Suresh and Jain, A. 2012. Preliminary phytochemical screening of medicinal plant *Ziziphus mauritiana* Lam. fruits. *Int. J. Curr. Pharm. Res.* 4 : 160-162.

Savithramma, N., M. R. Linga and Suhrulatha, D. 2011. Screening of medicinal plants for secondary metabolites. *Middle-East J. Sci. Res.* 8: 579-584.

Sen, A. and Amla, B. 2012. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia Azedarach* L. *Curr. Pharm. Res.* 4: 66 – 73.

Sofowora, A. 1993. Medicinal plants and traditional medicine in Africa. Spectrum books Ltd, Ibadan, Nigeria. 74:191- 289.

Szabo, M.A., G.Z. Varga, J. Hohmann, Z. Schelz, E. Szegedi, L. Amaral, and Molnar, J. 2010. Inhibition of quorum-sensing signal by essential oils. *Phytotherapy research.* 24: 782-786.

Tamatcho Kweyang, B.P., P.R. Fotsing Kwetche, M.E. Nougang, S.H. Zebazé Tougouet and Njiné, T. 2012. Species Richness and spatial distribution of pathogenic *Vibrio* (Bacteria, *Vibrionaceae*) in tropical surface waters: Yaoundé Mertropolis case (Cameroon, Central Africa). *Curr. Res. J. Biol. Sci*. 4: 584-591.

Tamil Selvan, A. 2013: Use and Abuse of Antibiotics, *I .J .P. R.S*, 2: 129-131.

Wendakoon, C., P. Calderon and Gagnon, D. 2012. Evaluation of selected medicinal plants extracted in different ethanol concentrations for antibacterial activity against human pathogens. *J.M.A.P.* 1: 60-68.

WHO, 2003. World Health report-Sharing the future. World Health Organization, Geneva, Switzerland. Retrieved from: www.who.int/whr/2003/en/overview (Accessed on: August 22, 2014).

Yorita-Christensen, K.L., R.C. Holman, C.A. Steiner, J.J. Sejvar, B.J. Stoll, and Schonberger L.B. 2009. Infectious diseases hospitalizations in the United States. *Clin. Infect. Dis*. 49: 1025-1035.

.