



E-ISSN: 2278-4136

P-ISSN: 2349-8196

JPP 2014; 3(4): 190-195

Received: 05-09-2014

Accepted: 15-10-2014

Guy Raymond Feuya Tchouya

Department of Chemistry, Faculty of Science, Scientific and Technical University of Masuku, Box. 223(Potos), Franceville, Gabon

Giresse Delphang Nguema Obiang

Department of Chemistry, Faculty of Science, Scientific and Technical University of Masuku, Box. 223(Potos), Franceville, Gabon

Edouard Akono Nantia

Department of Biological Science, Faculty of Science, University of Bamenda, Box. 39, Bamilli, Cameroon

Jacques Lebibi

Department of Chemistry, Faculty of Science, Scientific and Technical University of Masuku, Box. 223(Potos), Franceville, Gabon

Correspondence:**Guy Raymond Feuya Tchouya**

Department of Chemistry, Faculty of Science, Scientific and Technical University of Masuku, Box. 223(Potos), Franceville, Gabon.

Email: gfeuya@yahoo.fr

Phytoche, antimicrobial and antioxidant evaluation of the stem bark of *Thomandersia hensii* (Acanthaceae).

Guy Raymond Feuya Tchouya, Giresse Delphang Nguema Obiang, Edouard Akono Nantia, Jacques Lebibi

Abstract

The methanolic extract of the stem bark of *Thomandersia hensii* (Acanthaceae), was screened for its phytochemical constituents, *in vitro* antioxidant and antimicrobial activities using standard procedures. The results revealed the presence of alkaloids, polyphenols, sugars and steroids. *T. hensii* extract showed significant 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity ($IC_{50} = 0.1343 \pm 0.0262$ $\mu\text{g/ml}$), and polyphenol content (118.08 ± 0.95 mg/g of extract). Chromatographic separation of the extract led to the isolation of four compounds among which two were identified to bergenin and sitosterol 3-O- β -D-glucopyranoside. *In vitro* antimicrobial activities of the extract and identified compounds showed growth inhibition of *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Shigella flexneri*, *Salmonella typhimurium* and *Candida albicans*. Also, bergenin exhibited antibacterial activity against an amoxicilline resistant *P. aeruginosa* strain. This study showed that *T. hensii* extract is rich in phenolic compounds, contains bergenin and sitosterol 3-O- β -D-glucopyranoside, and possess antioxidant and antimicrobial activities.

Keywords: *Thomandersia hensii*; medicinal plant; phytochemistry; antioxidant activity; antimicrobial activity.

1. Introduction

The greater part of death due to infectious diseases as pneumonia, tuberculosis, measles, HIV/AIDS, malaria and diarrhea occur in developing countries. In these countries, people are not capable to insure health expenditure, due mostly to their extreme poverty^[1].

This situation becomes more alarming since there is the emergence of resistant microbial strains that compromise therapies based on conventional drugs^[2].

With such obstacles in the use of available antimicrobials, it is essential to look for new sources of cheaper, and more efficient drugs with broad spectrum of action.

One strategy of this research is to explore the plants used in traditional medicine. Indeed, the use of therapeutic plants (herbal medicine) is very old and currently WHO estimates that for the fight against many diseases, about 80% of the African population still uses traditional medicine for which the majority of therapies involve the usage of medicinal plants extractives^[3]. It seems clear that plant species with such great importance for the health of populations be subjected to scientific evaluation in order to ensure their better use.

In this paper, we present the phytochemical study and antimicrobial evaluation of the stem bark of *Thomandersia hensii*, an African medicinal plant of the Acanthaceae family.

Thomandersia hensii is a shrub of 1 to 4 m height (Fig. 1) that grows in the dense forests of Cameroon, Gabon, South west of Nigeria and the Democratic Republic of Congo.

A decoction of the leaves is used in Cameroon, Gabon and the Democratic Republic of Congo in the treatment of diarrhea. An infusion of the roots is used in some cases of urinary tract infections, intestinal parasites and as a tonic in cases of weakness and tiredness^[4]. The methanol extract is active against *P. falciparum*^[5].

The study of this plant is justified by the fact that it is not only widely used in the African traditional medicine, but also because to date, it has not been submitted to any phytochemical study.

The aim of our study was to extract, isolate, purify and characterize the different chemical constituents of the stem bark of *T. hensii*, and subsequently evaluate the free radical scavenging and the antimicrobial activities of the raw extract and isolated molecules.



Fig 1: *T. hensii* plant parts: (a) = leaves; (b) = trunk.

2. Material and methods

2.1. Plant material

Stem bark of *Thomandersia hensii* De Wild and Th. Dur (Acanthaceae) was collected in February 2012 from the Mondah forest near Libreville, in the Estuaire Region (Gabon). The plant was identified, and a Voucher specimen (N° 2098) was deposited in the National Herbarium of Gabon, Libreville.

2.2. Extraction

The stem barks of *T. hensii* were dried and ground. The powder obtained (932 g) was extracted at room temperature with hexane (1.5 L, 2 x 72 hours). The filtrate (1) obtained was evaporated, resulting in 1.5 g of hexane extract. The defatted material obtained after hexane extraction (745 g) was then extracted at room temperature with methanol (MeOH) (1.6 L, 2 x 72 hours). The filtrate (2) obtained was evaporated, resulting in 21.73 g of methanol extract.

2.3. Chemical screening

The plant extract was screened for its qualitative chemical composition, using standard methods described in the literature [6-7]. The identification of the following groups was considered: alkaloids, flavonoids, phenols, reducing sugars, saponosides, sterols-triterpenes and sugars.

Alkaloids 0.5 g of each extract was agitated with 5 ml of hydrochloric acid in a steam bath, then 1 ml aliquots of filtrate were treated with a few drops of Mayer's reagent or Dragendorff's reagent. The presence of a precipitate after treatment with either reagent is a preliminary indicator of the presence of alkaloids. To remove non-alkaloid compounds that could lead to false-positive reactions, part of the extract was alkalized with 40% ammonia solution then treated twice with chloroform. The second chloroform extract was concentrated and then retested with the Mayer and Dragendorff reagents. **Flavonoids** were detected by using the Shibata reaction or cyanide test. Briefly, 3 ml of extract was evaporated and the residue was dissolved in 2 ml of 50% methanol, then a few magnesium shavings and a few drops of concentrated hydrochloric acid were added. The development of a red-orange or purplish color indicates the presence of flavones aglycones. **Polyphenols and tannins** Boiled aqueous extract (1 ml) was mixed with 1% ferric chloride. A black-blue color indicates the presence of gallic tannins and a dark green color tannin catechist. When both were detected in the same extract, they were separated with Styasny's reagent. A drop of the extract was placed on a slab of silica gel and eluted in an atmosphere saturated with

chloroform/acetic acid/formic acid (5:4:1). Then the plates were sprayed with 10 ml of methanol solution at 5% nitrous acid and heated in an oven at 80 °C for 10 min. The presence of tannins is revealed by the appearance of blue spots, while polyphenols are revealed by a violet-blue, pink-orange, pink-violet, or red coloration. **Reducing sugars** One milliliter of extract was dissolved in 2 ml of distilled water and 1 ml of Fehling liquor and boiled for 30 min. The formation of a brick-red precipitate indicates the presence of reducing sugars. **Saponosides** 1% of each sample decoction was returned gradually in 10 ml test tube for a final volume of 10 ml. After two vigorous shakes, the tubes were left to stand for 15 min and the height of the foam was measured. The tube in which the height of the foam was at least 1 cm, showed the presence of saponosides. However, the height of the foam indicated the value of the foam index. **Sterols and triterpenes** These families of compounds were identified by using the Lieberman–Burchard reaction. Briefly, 0.5 g of extract was dissolved in 0.5 ml of chloroform in 0.5 ml of acetic anhydride, and cooled on ice before carefully adding sulfuric acid. A change in color from purple to blue indicates the presence of sterols, while a green or purple-red color indicates the presence of triterpenes. **Sugars:** a little quantity of the extract was dissolved in an ethanol/ α -naphthol (99%/1%) solution contained in a test tube, then allowed to run on the tube wall few drops of concentrated sulphuric acid. Sugars presence was detected by the emergence of a red ring at the interface.

For all the families tested, according to the precipitation or color intensity of each tube, following evaluations were given: (+++); (++) ; (+).

2.4. Determination of free radical scavenging activity

The antiradical activity of *T. hensii* extract was determined according to the method described by Nantia *et al.* [8]. Briefly 980 μ l of freshly prepared DPPH solution (40 μ g/ml) was introduced in tubes and the extract or standard vitamin C (0.02, 0.2, 2, 20, and 200 μ g/ml) were added. After 30 min the change from the radical to the non-radical form leads to the disappearance of the purple coloration of DPPH, which was recorded by spectrophotometry at 517 nm. The inhibitory potential of extracts was expressed through their inhibitory concentration fifty (IC₅₀).

2.5. Determination of total phenolic content

The amount of total phenolics in *T. hensii* extracts were determined with Folin–Ciocalteu reagent according to the method of Singleton and Rossi [9], with slight modification using gallic acid as a standard. Briefly, in 200 μ l of extract (2 μ g/ml) was added 500 μ l of 1/10 diluted Folin reagent and 20 % Na₂CO₃. The mixture was allowed to stand for 30 min with intermittent shaking, and the absorbance was measured at 730 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). The total phenolic content was determined as mg of gallic acid equivalent per gram of plant extract using an equation obtained from the standard gallic acid calibration graph.

2.6. Fractionation and purification

15.77 g of the methanol extract obtained (21.73 g, 2.33%) was submitted to flash chromatography on a 70-230 mesh silica gel column (270 g) with stepwise gradient elution by n-hexane/EtOAc mixtures (100:0; 80:20; 50:50; 0:100), and

EtOAc/MeOH mixtures (50:50; 0:100). Twenty nine column fractions, each containing 100 ml were collected and combined according to their TLC profiles on precoated Kieselgel 60 F₂₅₄ plates developed with CH₂Cl₂/MeOH mixtures. Four groups of fractions F1 (1-5), F2 (6-13), F3 (14-20) and F4 (21-29), respectively, were eluted. The fraction F1 obtained in a very little quantity (1.2 mg) was not studied. Fraction F2 (11.2 g) was subjected to column chromatography over Si gel (70-230 mesh) eluting with CH₂Cl₂/MeOH gradient of increasing polarity resulting in the isolation of four compounds: (G1) (2.2 mg), (G2) (15 mg), (G3) (20 mg) and (G4) (1.5 mg). Fraction F3, after concentration and chromatography over Si gel eluting with

petroleum ether/EtOAc (85:15) gradient of increasing polarity afforded (G2) (8 mg), (G3) (2.0 mg) and (G4) (0.5 mg). Fraction F4 appeared on the thin layer chromatography (TLC) plate under different system as a very complex mixture and so, due to its quantity, was abandoned.

Compounds **G2** and **G3**, after spectroscopic analysis and comparison of their spectroscopic and physical data with those reported in the literature ^[10-11], were identified respectively to bergenin and sitosterol 3-*O*-β-*D*-glucopyranoside (Fig. 2). The extraction scheme is shown below (Fig. 3).

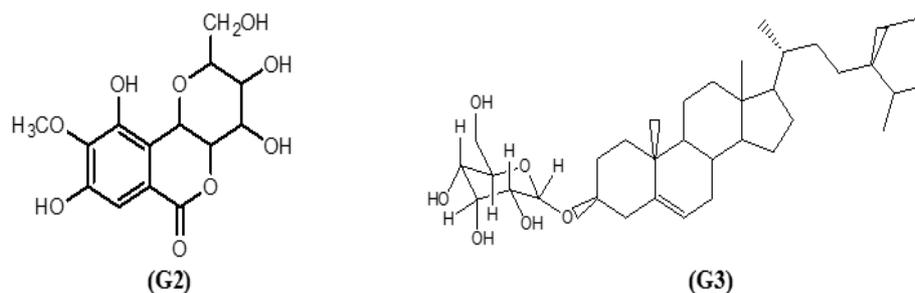


Fig 2: Chemical structures of isolated and identified compounds.

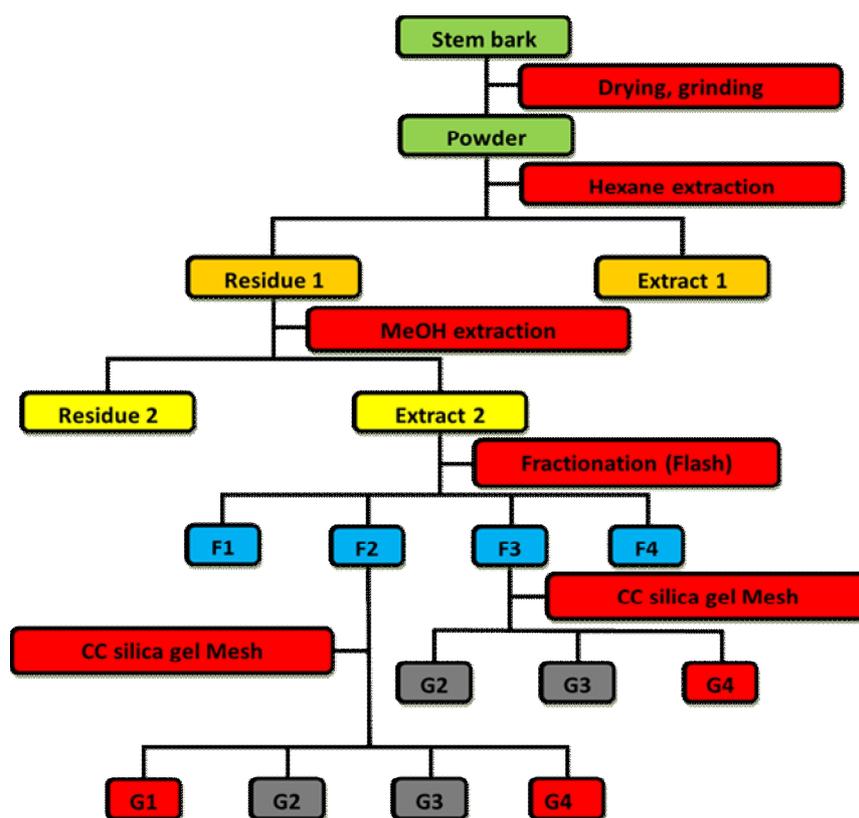


Fig 3: Extraction and fractionation scheme of the stem bark of *T. hensii*.

2.7. Microbiological surveys

2.7.1. Microorganisms

Seven bacterial species, Gram-positive (*Staphylococcus aureus* and *Staphylococcus Saprophyticus*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*,

Pseudomonas aeruginosa, *Salmonella typhimurium* and *Shigella flexneri*), and the yeast *Candida albicans* obtained from “Centre Pasteur du Cameroun”, were used for the antimicrobial tests. The strains were maintained on agar slant at 4 °C in the laboratory, where the antimicrobial tests were

performed. The strains were activated at 37 °C for 24 h on Tryptica soy agar or Sabouraud glucose agar supplemented with chloramphenicol respectively for bacteria and fungi prior to any screening. Mueller Hinton agar and Brain Hearth Infusion (BHI) were used for the antimicrobial assays [12].

2.7.2. Antimicrobial activity

The antimicrobial activities of the extracts and isolated compounds were determined using the Berghe and Vlietnick agar-well diffusion method [13]. Plates containing 30 ml of sterile nutrient broth were inoculated with standardized inoculate prepared using a cell suspension of about 1.5×10^6 CFU/ml "colony forming units per milliliter". Four wells of 5 mm diameter were made on each plate with sterile Pasteur pipette and 50 µl of the plant extract and isolated molecule at a concentration of 20 mg/ml was dispensed into each well. The sample was allowed to diffuse into the medium for 1 hour at room temperature and then, it was incubated for 24 hours at 37 °C. The zones of growth inhibition were measured and recorded in millimeter. The negative control was set up in a similar manner except that the extract was replaced with sterile distilled water. All the testing was done in duplicate. For the positive control, two reference antibiotics (amoxicillin and gentamycin) were used in a similar manner and at the same concentration (20 mg/ml).

2.8. Data analyses

Data were expressed in mean \pm standard deviation, and for the anti-radical activity, the inhibitory concentration fifty (IC₅₀ value is the amount of the antioxidant required to decrease the initial DPPH radical concentration to 50%) of the extract was determined using Graph Pad Prism software. Differences between fractions assessed by one factor ANOVA followed by the Student-Newman-Keuls test.

3. Results

3.1. Qualitative analysis of phytochemical screening

Phytochemical screening using qualitative analysis on the stem bark of *T. hensii* methanol extract showed the presence

of the following constituents: alkaloids, polyphenols, sugars and terpenoids. However, flavonoids, reducing sugars and saponosides were not detected in the plant. The details on phytochemical screening results are shown in Table 1.

Table 1: Qualitative analysis of phytochemical constituents of the stem bark of *T. hensii*.

Phytochemical constituents	Stem bark extract
Alkaloids	++
Flavonoids	-
Phenols	+++
Reducing sugars	-
Saponosides	-
Sugars	+++
Triterpenes /Sterols	+++

+++ Very intense, ++ intense, + weak, - absent.

3.2. Chromatographic and antimicrobial analysis

Chromatographic separation of the stem bark of *T. hensii* (Acanthaceae) led to the isolation of four compounds, among which two were identified to known bergenin (**G2**) and sitosterol 3-*O*- β -*D*-glucopyranoside (**G3**).

The antimicrobial activities of the extract and isolated compounds were evaluated against some pathogenic strains using the agar-well diffusion method. Inhibition diameters obtained are shown in Table 2. *T. hensii* significantly inhibited the growth of *E. coli* (ID= 8 mm), *P. aeruginosa* (ID= 8 mm), *S. aureus* (ID= 8 mm), *S. saprophyticus* (ID= 10 mm), *S. flexneri* (ID= 11 mm), *S. thyphimurium* (ID= 8 mm); while it has no effect on *K. pneumoniae*, and *C. albicans*. Among different microorganisms, purified compounds sitosterol 3-*O*- β -*D*-glucopyranoside (**G3**) and in some extent bergenin (**G2**) selectively inhibited the growth of *P. aeruginosa*, *S. aureus* and *S. flexneri*. This suggest that *T. hensii* has a large antimicrobial spectrum than the purified compounds, bergenin and sitosterol 3-*O*- β -*D*-glucopyranoside.

Table 2: Inhibition diameters of the extract and isolated compounds.

Microorganisms	Inhibition diameters (ID in mm)				
	Extract	G2	G3	Amoxicillin	Gentamicin
<i>E. coli</i>	8	0	0	8	17
<i>P. aeruginosa</i>	8	12	8	-	26
<i>K. pneumoniae</i>	-	0	0	-	-
<i>S. aureus</i>	8	0	8	8	26
<i>S. saprophyticus</i>	10	0	0	-	-
<i>S. flexneri</i>	11	0	8	14	20
<i>S. thyphimurium</i>	10	0	0	19	26
<i>C. albicans</i>	-	0	0	-	-

3.3. Total phenolic content and radical scavenging effect

The methanol extract of the stem bark of *T. hensii* exhibited a total phenolic content of 118.08 ± 0.95 mg/g extract, and a DPPH radical scavenging activity (IC₅₀) of 0.1343 ± 0.0262 µg/ml, significantly lower ($P < 0.001$) than the reference compound, vitamin C (0.267 ± 0.009 µg/ml).

4. Discussion

The chemical screening that we did, revealed that *T. hensii* contained biomolecules potentially active against some bacterial and fungal pathogenic strains. These included triterpenes and sterols, which play an important function in healing scar and wound, and that have been shown to be cardiogenic and antiparasitic [14, 15]; polyphenols, that are antibacterials, antifungals and antiseptic; alkaloids which are

stimulants, antibiotics, antifungals and pest-destroying [14]. Sugars have antitussive, expectorant, analgesic, immunomodulatory and cytoprotective properties [14].

So from this observation, it can be possible to justify the common usage of *T. hensii* in traditional medicine for the treatment of several diseases by the presence in its stem bark of many potentially active biomolecules, including terpenoids, phenols and alkaloids. The confirmation of this came from the results obtained after *in vitro* antimicrobial analysis of the extract and isolated molecules. Hence, the crude extract has an inhibitory activity on the growth of *S. typhimurium*, *S. flexneri* and *S. saprophyticus*, with inhibition diameters of 10 mm, 11 mm and 10 mm respectively. In the same way, compound **G2** presented inhibitory activity on the growth of *P. aeruginosa* with an inhibition diameter of 12 mm. *P. aeruginosa*, *S. aureus* and *S. flexneri* presented a very weak sensitivity vis-à-vis compound **G3**, as well as *E. coli*, *P. aeruginosa* and *S. aureus* for the crude extract. Overall, *C. albicans*, *K. pneumoniae* and *S. saprophyticus* proved insensitive to **G2** and **G3**, amoxicillin and gentamycin. However, the diameters of inhibition induced by **G2**, **G3** and amoxicillin remain lower than those of gentamycin for all strains. Nevertheless, it is important to realize that compound **G2** shows a good inhibitory activity on *P. aeruginosa*, a strain resistant to amoxicillin which is a reference antibiotic.

Traditionally, this plant is used to treat some infectious diseases such as diarrhea. Therefore, inhibition of the growth of *P. aeruginosa*, a bacteria responsible for diarrhea [16], allows to provide a rationale ethnobotanic use of this plant. Compound **G2**, with its good antibacterial activity on *P. aeruginosa*, even appeared, to be considered as the anti-diarrheal active principle of the stem bark of *T. hensii*.

It has been shown that plant tissues contain a variety of bioactive compounds with antioxidant activity and have various therapeutic effects [15]. Phenolic compounds have been reported as antioxidant agents [15]. Phenolic compounds were found in the stem bark of *T. hensii*. Phenols are known to act as antioxidants, which can neutralize unstable and reactive molecules, they could protect membrane lipids from oxidation [15].

In this study, the investigation of the free radical scavenging ability of the stem bark of *T. hensii*, was performed by the DPPH scavenging assay. DPPH can measure the ability of antioxidant compounds to scavenge free radical by converting from deep violet colour to discolouration. On the other hand, DPPH assay is a relatively stable free radical [17-18].

This study showed that, the stem bark of *T. hensii* methanol extract has higher antioxidant activity than that of vitamin C. The antimicrobial activity of *T. hensii* extract could be due to its phenolic content and anti-radical activity.

5. Conclusion

Altogether *T. hensii* stem bark methanolic extract is rich in phenolic compounds, contains bergenin and sitosterol 3-O- β -D-glucopyranoside, and possess antioxidant and antimicrobial activities. It can be considered as an important source of natural antioxidant and antimicrobial compounds. This partially validates the use of this species in the treatment of some infectious diseases such as diarrhea in traditional medicine.

6. Acknowledgements

We are very much grateful to local informants and healers who shared their knowledge on the use of medicinal plants with us. The authors thank Mr Thomas NZABI, at the National Herbarium of Gabon, Libreville, Gabon. We are also grateful to the "Centre Pasteur du Cameroon" for the supply of microbial strains. This work was supported by the financial assistance of the International Foundation for Science (IFS) through the grants N° F-4738 "1 & 2" (Dr. Feuya Tchouya).

7. References

1. WHO. Removing obstacles to healthy development: report on infectious diseases. World Health Organization, Geneva, 1999, 12-14.
2. Vanden BDA, Vlietinck AJ. Screening methods for antibacterial and antiviral agent from higher plants. Academic Press, London, 1991, 47-58.
3. WHO. Traditional Medicine Strategy 2002-2005. World Health Organization, Geneva, 2002, 1.
4. Burkill HM. The Useful Plants of West Tropical Africa. Vol.1, Royal Botanic Garden, Kew, 1985, 28.
5. Bickii J, Feuya TGR, Tchouankeu JC, Tsamo E. Antimalarial Activity in Crude Extracts of some Cameroonian Medicinal Plants. African Journal of Traditional, Complementary and Alternative medicines 2007; 4(1):107-111.
6. Mengome LE, Feuya TGR, Eba F, Nsi EE. Antiproliferative effect of alcoholic extracts of some Gabonese medicinal plants on human colonic cancer cells. African Journal of Traditional, Complementary and Alternative medicines 2009; 6(2):112-117.
7. Mengome LE, Akue JP, Souza A, Feuya TGR, Nsi EE. *In vitro* activities of plant extracts on human *Loa loa* isolates and cytotoxicity for eukaryotic cells. Parasitology Research 2010; 107:643-650.
8. Nantia AE, Manfo TFP, Beboy ESN, Moundipa FP. *In vitro* antioxidant activity of the methanol extract of *Basella alba* L. (Basellaceae) in rat testicular homogenate. Oxidants and Antioxidants in Medical Science 2013; 2(2):131-136.
9. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture 1965; 16:144-158.
10. Jahodar I, Kolb I, Lioka A. NMR spectral analysis of bergenin. Fitoterapia 1992; 63(3):260-261.
11. Ramaiah PA, Lavie D, Budhirija RD, Sudhir S, Garg KN. Spectroscopic studies on a withanolide from *Withania coagulans*. Phytochemistry 1984; 23:143-149.
12. National Committee for Clinical Laboratory Standard (NCCLS). Performances standards for antimicrobial disc susceptibility tests. Edn 4, Vol. 20, NCCLS. M2 A4, Villanova, 1990, 1-28.
13. Berghe VA, Vlietinck AJ. Screening methods for antibacterial and antiviral agents from higher plants. In: Hostettmann, K. (Ed.), Methods in Plant Biochemistry, Vol. 6, Academic Press Limited, London, 1991, 47-69.
14. Bruneton J. Pharmacognosie, Phytochimie, Plantes médicinales. Edn 3, TEC & DOC, Paris, 1999, 3-799.
15. Aziz AFB, Iqbal M. Antioxidant activity and phytochemical composition of *Cynometra cauliflora*. Journal of Experimental and Integrative Medicine 2013;

3(4):337-341.

16. Fauchère JL, Avril JL. Bactériologie générale et médicale. Ed. 3, Ellipses, Paris, 2002, 27-29.
17. Shah MD, Hossain MA. Total flavonoids content and biochemical screening of the leaves of tropical endemic medicinal plants *Merremia borneensis*. Arabian Journal of Chemistry 2011.
18. Hossain MA, Shah MD, Gnanaraj C, Iqbal M. *In vitro* total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant *Tetrastigma* from Sabah. Asian Pacific Journal of Tropical Medecine 2011; 717-721.