Phytochemical analysis and Antimicrobial activity of *HAGENIA ABYSSINICA*

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Abstract

Introduction: Ethiopian communities highly depend on local plants to safe and sound their survival and health. Local trees are subjugated and used intensively for medicinal uses.

Objective: The aim of the present study was too carried out phytochemical analysis of organic extract of *Hagenia abyssinica and to find* out antihelmentic property of *Hagenia abyssinica.*

Methods: *Hagenia abyssinica* female flower extracts was used for plant component analysis and for determination of antihelmentic activity. *Earth worm (Pheretima posthuma)* strains were used for experimental purpose. Disc diffusion method was used to assess the antibacterial effect of the extracts on micro-organisms.

Results: The phytochemical screening indicated the presence of flavonoids, tannins, steroid, alkaloid, saponins in all extracts. Antibacterial and antihelmentic activity of Ethanolic, Methanolic, Hexane and Petroleum Ether extract of *Hagenia abyssinica* female flowers was highly active against Adult earthworm (*Pheritima posthuma)* and active against *Staphyloccoccus aureus* and showed less activity against *Salmonella typhi*.

Conclusion: The present study finally demonstrates that *Hagenia abyssinica* is a good source of various phytochemical such as Saponins, Phlobathanins, Flavonoids, Anthraquinones, Phenols, Terpenoids, Alkaloids, Steroids, Glycosides, Tannins. The antibacterial activity *Hagenia abyssinica* was clearly shown by the present study against bacteria. All these preliminary reports affirm an in depth analysis of the usefulness of *Hagenia abyssinica* as miracle drug against various ailments.

Keywords: Antimicrobial activity; *Hagenia abyssinica* ; Phytochemicals; Traditional medicine

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Introduction

Hagenia abyssinica in the vicinity known in East Africa as 'Kosso', is the species and it is a monospecific genus and is most intimately interrelated to the monospecific genus Leucosidea. The specific name means 'from Ethiopia'. The dioecious plant is a characteristic tree of the Afro-Montane Forests of Eastern Africa. In Ethiopia dried female flowers are traditionally used as an anthelmintic remedy, particularly for tapeworm (Assefa *et al.,* 2010). Infections with *Taenia saginata* are common in the country due to the regular traditional consumption of raw meat (Abegaz *et al.,* 1999). Historically it was one of the most famous African plants that were included in the European pharmacopoeia based on the description of a Portuguese priest in 1645 of the usage as vermifuge by Ethiopians (Abegaz et al., 1999).

H. abyssinica shows a range of adverse effects. The plant possibly causes optic atrophy. A study with chicks indeed indicated retinal pathology and defects in visual behavior (Rogers *et al.,* 1985). In traditional medicine the extract is also used as abortifacient which initiated investigations examining it as a potential contraceptive agent (Farnsworth *et al.,* 1975).

Traditionally the dried female Kosso flowers are used as decoction or suspension in water. The powdered material is usually macerated with water. Children infested with the parasite resist taking the drug and are sometimes punished by their parents in order to force them to drink concoction. Although the non-polar kosotoxin is believed to be the active principle, some contributing components may well exist in the crude water extract of *Hagenia abyssinica* and influence its activity (Woldemariam *et al.,* 1992). The occurrence of glycosides possessing a variety of biological activities, have been reported from other natural sources (Singh and Bharate, 2006). Therefore, we investigated for the first time the polar and non-polar constituents of *Hagenia abyssinica* female flowers.

Present work was conducted to screen type of phytochemical constituents present in and antihelmentic potential of various extracts of *Hagenia abyssinica* female flowers in earth worms (*Pheretima posthuma)* some and pathogenic bacteria.

Materials and Methods

Collection of plant materials: The experiments were conducted from December, 2015 to June 2016 at Bless Agri Food Laboratory Services PLC, Addis Ababa. Female flowers *Hagenia abyssinica* plants were collected from three different weredas in Gurage zone. It was ensured that the plant was healthy and

uninfected. The female flowers were thoroughly dried under shed.

Fig. 1: Female *Hagenia abyssinica* tree and its flowers

Kosin extraction: Two hundred grams (200g) of dry plant materials (*Hagenia abyssinica*) were macerated in 1600ml of 70% ethanol for 72hours with intermittent shaking in duplicates; Similarly, 200g of fresh dried materials (*Hagenia abyssinica*) of the study plants were soaked in 1.6 liters of methanol with intermittent shaking for 72 hours; Two hundred fifty grams (200g) of dry plants material (*Hagenia abyssinica*) were macerated in 1600ml of n-hexane for 72hours with intermittent shaking in duplicates and Two hundred fifty grams (200g) of dry plants material (*Hagenia abyssinica*) were macerated in 1600ml of petroleum ether for 72hours with intermittent shaking in duplicates. Filtrations through cotton wool were done to remove coarse particles and finely through filter paper (Whatman®, England). The filtrates were concentrated on Rota-vapor type Buchi-R, Switzerland under reduced pressure at 40^0C. The extracts were transferred to previously weighed kidney and petri dishes and put into an oven to dry completely at 50^0C to produce solid materials. The mean yield of the duplicate samples were determined and recorded. Thereafter dried extracts were packed into universal bottles and kept at 4^0C till needed for bioassay tests.

Phytochemicals Screening on extracts from flowers of *H. abyssinica:* There were no previous phytochemical reports on the flowers of the plant. To the best of our knowledge the preliminary phytochemical screening for the flowers of *H. abyssinica* were carried out to analyze the presence of compounds namely: Saponins, Anthraquinones, Flavonoids, Phenols, Alkaloids, Tannins, Terpenoids, Steroids, Phlobatannins and Glycosides.

Test for Saponins: To 0.5 g of each crude extract 5 mL of distilled water were added and shaken and then were heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins (Egwaikhide and Gimba, 2007).

Test for Anthraquinones: To 0.5 g of each crude extracts 10 mL of benzene were added and shaken and then filtered. 0.5 mL of 10% ammonia solution were added to the filtrate and the mixture were shaken well and the presence of the violet color in the layer phase indicated the presence of the anthraquinones (Evans', 1996).

Test for flavonoids: To 0.5 g portion of crude extract 10 mL of ethyl acetate were added and heated with a steam bath for 3 min. The mixtures were filtered and 4 mL of the filtrate were shaken with 1 mL of dilute ammonia solution and a yellow coloration were observed (Sofowora, 1993).

Test for Phenols: 0.5 g of each of crude extracts were put in a different test tube and treated with a few drops of 2% of $FeCl_3$; bluish green or black coloration were indicated the presence of phenols (Harborne, 1998).

Test for Alkaloids: 0.5 g of crude extract was defatted with 5% ethyl ether for 15 min. The defatted samples were extracted for 20 min with 5 mL of aqueous HCl on a boiling water bath. The resulting mixtures were centrifuged for 10 min at 3000 rpm. 1 mL of the filtrates were treated with few drops of Mayer's reagent and a second 1 mL with Dragendroff's reagent and turbidity were observed (Wagner *et al*, 1984).

Test for Tannins: 0.5 g of each crude extracts were boiled in 10 mL of water in a test tube and then were filtered. A few drops of 0.1% ferric chloride were added to give brownish green or a blue black coloration (Ayoola *et al.*, 2008).

Test for Terpenoids: 0.5 g of each crude powders were separately dissolved in 5 mL of methanol. 2 mL of the extract were treated with 1 mL of 2, 4-dinitrophenyl hydrazine were dissolved in 100 mL of 2M HCl. Yellow-orange colorations were observed as an indication of Terpenoids (Sofowora, 1993).

Test for Steroids: 0.5 g of each crude extracts were dissolved in 5 mL of methanol. 1 mL of the extract were treated with 0.5 mL of acetic acid anhydride and cooled in ice. This mixed with 0.5 mL of chloroform and 1 mL of concentrated sulphuric acid was then added carefully by means of a pipette. At the separations level of the two liquids, reddish-brown rings were formed, as indication of the presence of steroids (Harborne, 1998).

Test for Phlobatannins: 2 mL of each extracts were added to 2 mL of 1% HCl and the mixtures were boiled. Deposition of a red precipitates were taken as an evidence for the presence of phlobatannins. (Njoku

and Obi, 2009)

Test for Glycosides: 0.5 g of crude extracts were dissolved separately in 5 mL of methanol. 10 mL of 50% HCl were added to 2 mL of each extract in test tubes. The mixtures were heated in a boiling water bath for 30 min. 5 mL of Fehling's solution were added and the mixtures were boiled for 5 min to give a brick red precipitate as an indication for the presence of glycosides (Harborne, 1998).

The organisms used were *Pheritima posthuma*, *Staphylococcus aureus and Salmonella typhi*. The organisms were obtained from ambo university biology laboratory and maintain according to specification.

Antihelmentic activity: Adult earthworm *Pheritima posthuma* were choosen (due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being) from moist soil, obtained from agricultural fields in Ambo University. Three test groups were taken each containing six earth worms of approximately equal size (8±1 cm). Albendazole was taken as standard drug and different concentrations (25 mg/mL, 50 mg/mL, 100 mg/mL and 200 mg/mL) were prepared in normal saline containing 5% DMF. The *H. abyssinica* extracts of different concentrations were prepared by dissolving in minimum quantity of DMF and making up to the final volume with normal saline to obtain 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL concentrations. One of the groups is taken as control group, which was treated with normal saline containing 5% DMF. Paralysis onset time and death time of individual worms were noted. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility followed by fading away of color of worm.

Antibacterial activity: The **antibacterial** activity of the *Hagenia abyssinica* female flower extracts was determined using agar well diffusion method by following the known procedure. Small amount of diluted bacteria suspension were poured over the media to spread uniformly on the surface. Later when the surface was little dried wells of 8mm were punched in the agar with stainless steel borer and filled with 300µl of plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 32°C for 16-18 hours and the antibacterial activity was assessed by measuring the diameter of the zone of inhibition. The antibacterial activities of the extracts were evaluated by comparing their zones of inhibition with standard antibiotic Chloraphenicol.

Result and Discussion

The extraction yield is a measure of the solvent efficiency to extract specific components from the original plant powder. The percentage yield of crude extract in respective solvent was recorded in Table 2. It could be calculated according to the method as follows (Zhang *et al*., 2007).

Table 1: Comparison on yield of each *Hagenia abyssinica* crude extract at different solvents

Solvents	Yield in %
Methanol	33
Ethanol	18
n- haxene	11
Petroleum ether	19

During the present work the aim is achieving pure compound but not determining the highest yield of extract. As a result, the researchers preferred the maceration method to minimize the possibility of thermal decomposition of thermo-labile components from the plants. The percent yield from methanol as main solvent is better than other solvents. On comparing the yields of plant material on the different extraction solvents, the highest yields were observed on methanol extracts while the lowest on hexane extracts. Non polar compounds were extracted using hexane and petroleum ether while polar compounds were extracted using methanol and ethanol. Hence, suggesting that the selected plants had abundant polar compounds compared to non- polar compounds.

Phytochemical Analysis: The world is rich in natural and unique medicinal plants. Medicinal plants are now getting more attention than ever because they have potential of enormous benefits to all mankind, especially in the line of phytomedicine. Phytomedicine represents one of the most important fields of traditional medicine all over the world and are of primary importance to the health of individuals and communities. The medicinal value of traditionally important plant species is due to presence of some chemical substances which produce a definite physiological action on human body like alkaloids, tannins, flavonoids, saponins, etc (Edeoga *et al*., 2005; Khan *et al*., 2011). To promote the proper use of phytomedicine and to determine their potential as sources for new drugs, it is essential to study phytochemical constituents present in the plant species in order to support species from traditional location to world medicinal plants category.

In the present study the qualitative analysis of *H. abyssinica* female flower extracts were carried out for dried plant samples. The preliminary phytochemical screenings on flower (methanol, ethanol, petroleum ether and n-haxene) extracts were tested in this study. Saponins, tannins, alkaloids, flavonoids, terpenoids, phlobathanins and phenolic compounds were revealed to be present in extracts of *Hagenia abyssinica* which are known to have antimicrobial activity (Table 3). This shows that the plant constituents which might have high level of medicinal values (Oloyed, 2005; John *et al*., 2011). This could be responsible for the versatile medicinal properties of the plant.

Phenolic compounds serve in plant defense mechanism to counteract reactive oxygen species in order to survive and prevent molecular damage by microorganisms, insects and herbivores (Sengul *et al*., 2009). Examples of known phenolic compounds with antimicrobial activity include benzoic acid, caffeic acid, cinnamic acid and gallic acid (Aljadi and Yusoff, 2003). Phenolic compounds were present in 75% of the extracts tested for phytochemicals.

Anthraquinone compounds are known for their antimicrobial activity with emodin and chrysophanol as examples (Ayo *et al*., 2007, Garcia-sosa *et al*., 2006), while drimanes are terpenes which are potent antimicrobials (Gershenzon and Dudareva, 2007). The antimicrobial activity observed in some of the tested plant extracts can therefore be attributed to the presence of these compounds in the plants used.

Table 3: Phytochemical screening of different crude extracts of leaves of *H. abyssinica*

No	Phytochemical	Solvents			
		Methanol extract	Ethanol extract	n- hexane	Petroleum ether
1	Saponins	+	+	-	+
2	Tannins	+	+	-	-
3	Flavonoids	+	+	+	-
4	Anthraquinones	-	-	+	-
5	Phenols	+	+	+	-
6	Terpenoids	+	+	+	+
7	Alkaloids	-	+	-	-
8	Steroids	+	-	-	-
9	Glycosides		-	-	-
10	Phlobathanins	+	-	ND	ND

+ = the presence of phytochemical constituents - = the absence of phytochemical constituents

Preliminary screening for phytochemicals in the plant extracts revealed presence of terpenoids and phenolics in both plants with anthraquinones present only in hexane extract of leaves of *Hagenia abyssinica*. Flavones and alkaloids were present in both plant extracts tested (Table 4). Methanol extract of female flowers of *Hagenia abyssinica* had the most phytochemicals. Terpenoids were present in almost all extracts tested, but were most abundant in *H. abyssinica* extracts while phenolic compounds were present in trace amounts. These secondary metabolites are known to be biologically active and play significant roles in bioactivity of medicinal plants, because the medicinal values of medicinal plant lies in these phytochemical compounds which produce a definite and specific action on the human body.

Alkaloids are naturally occurring chemical compounds containing nitrogen atoms. They often have pharmacological effects and are used as medications and drugs [Rhoades, 1979]. Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes [Korkina and Afanas'ev, 1997]. Plant terpenoids are used extensively for their aromatic qualities. They play role traditional herbal medicines and are under investigation for Antibacterial, Antineoplastic and other Pharmaceutical fun [Yamunadevi, *et al*., 2011]. Tannins have shown potential Antiviral, Antibacterial and Antiparasitic effects. Saponins cause hemolysis of red blood cells [Winter *et al.*, 1993]. The antihelmentic activity was screened because of their great medicinal properties towards the pathogenic organisms. The medicinal plant *Hagenia abyssinica* showed good antihelmentic against earth worms.

The presence of saponins, phenols and alkaloids could confer antibiotic property on the plant. This is supported by the findings of (Jacob and Burri, 1996). The natural tendency of saponins toward off microbes makes them good candidates for treatment of fungal and yeast infections (Eka, 1998). These compounds serve as natural antibiotics, which help the body to fight infections and microbial invasion (Sodipo *et al*, 2000).

Therefore, the phytochemical screening result reveals that the presence of these phytochemical constituents supports the use of *H. abyssinica* plant in folklore medications and it is probable that these phytochemicals are responsible for the healing properties of these plants which have been claimed by the peoples in the area of this study. It is recommended that the amount of each phytochemical component should be determined. In former times acylphloroglucinols from *Hagenia abyssinica* (Flores Koso, Rosaceae), Mallotus philippinensis (Kamala = Rottlerae glandulae) and Dryopterisfilix mas (Filicis rhizoma) have been used as taeniafuge.

Analysis of Antimicrobial Activities on *H. abyssinica:* Infectious diseases are the leading cause of death worldwide, accounting for nearly one half of all deaths in tropical countries which are also becoming a significant problem in all countries. Microbial infections are great challenge to human health concern and it is even exacerbated by the growing resistance to the conventional drugs (Ibezim, 2005). Thus, researchers have resort to find remedy from plants for

infectious diseases. Development of new antimicrobial agents is among the proposed solution to solve this problem. In this regard plants can be provided a good alternative in search for new chemicals with a wide range of antibacterial and anthelminthic activities (Casley-Smith, 1997).

H. abyssinica is traditionally known plant for deworming intestinal parasites and treating infections. Flowers of crude extracts, were taken to test the sensitivity *in vitro* against two bacterial and one helminthic species (Table 4).

Table 4: Zone of bacterial growth inhibition (mm) for crude extract, from *H. abyssinica flower and fruit*

Sample	Conc. In (mg/ml)	Types of bacteria with mean inhibition diameter (mm)	
		S. aureus	***S. typhi***
HAT-MT	100	21	15
	50	14	12
HAT-ET	100	20	14
	50	13	10
HAT-PE	100	14	12
	50	9	8
HAT-HEX	100	15	14
	50	10	9
Chloramphenicol	100	24	21

Value represents mean of three replications; HAT = *Hagenia abyssinica Tesfu*, MT = Methanol, ET = Ethanol

The antibacterial activity of these extracts was found to be selective. The largest zones of inhibition were recorded with methanol crude extract from *H. abyssinica* against *S. aureus* (21 mm) followed by *S. typhi* (15 mm). The antibacterial activities of other crude extracts were followed by ethanol extracts, and hexane respectively. The lowest activity was determined in a petroleum ether crude extract. As can be seen from Table 4, the entire test samples showed considerable antibacterial activity against the test organisms. As a whole, methanol crude extract showed maximum activity followed by ethanol, and hexane in respective order.

In general the four compounds, ethanol crude extract and methanol crude extract exhibited the significant activities against the two bacteria (*S. aureus* and *S. typhi*). The susceptibility of *S. aureus* and *S. typhi* to extracts demonstrated higher antibacterial activities in terms of growth inhibition of the test organisms.

The antibacterial tests showed that all of the test samples clearly indicate that a considerable antibacterial activity against the bacterial species used in the study. As a whole, methanol crude extract was found to be the most active extract for the tested bacteria except *Salmonella typhi* followed by ethanol. In general as concentration of sample increases the mean inhibition zone value increases. This might be due to the high concentration of antibacterial compounds.

The methanol, ethanol, and hexane extracts were significantly active against *S. aureus* and *S. typhi* at concentration of 100% and had inhibition zones of above 12 mm. Methanol extract in particular had the highest inhibition diameter of 21 mm on *S. aureus* and 15 mm on *S typhi*. The explanation of this activity could be due to high concentration of anti-bacterial compounds in these extracts. At 50% concentration except methanol and ethanol extract the rest had only mild effects on *S. aureus* and *S typhi*. This might be due to low concentration of anti-bacterial compounds in the samples. There is no inhibition effect on *Salmonella typhi* and *S. aureus* for petroleum ether and hexane extracts but for the rest two extracts it had only mild effects. As compared to standard drug the antibiotic activity of the h. abyssinica plant extracts on *S. aureus* and *S typhi* is 87.5% and 71.5%, respectively. This shows the plants as a good candidate for development of antibacterial drugs.

The commercial standard drug (Chloramphenicol) showed the greatest inhibition effect against in all bacteria rather than the tested samples, the control (solvents) has no inhibition zone and was not observed any effect to the test solutions.

In general the three compounds and volatile oil exhibited the significant activities against on the two bacteria (*S typhi* and *S. aureus*). This is the fact that the compounds and fractions make it a suitable candidate for investigation of in control of antibiotic on these two tested bacteria.

Results of the present investigation clearly indicate that the antibacterial activities vary with the test organisms, plant material and the solvents used. This is due to the difference in polarity of solvents is perhaps responsible for the difference in solubility of plants active principles. Thus, the result ascertains the value of plant used in the study could be of considerable interest to the development of new antimicrobial drugs.

In this study, all extracts of *Hagenia abyssinica* showed significant antibacterial activity against *S. aureus*; *S. typhi* Table 4. In comparison, polar extracts showed significantly better results than non-polar extracts. Our results are consistent with findings of other researchers [Hindumathy, 2011, Ojo and Anibijuwon, 2010; Mothana et al.,

2010; Duraipandiyan et al., 2006; Caccioni et al., 2000; Kraft and Hobbs, 2004] so that most plant extracts have inhibition effect on Gram positive bacteria and little effect on Gram negative bacteria. This inhibition effect can be related to its active compounds that include: steroids and terpenoids, alkaloids, flavonoids, tannins, phenolic compounds, and saponin [Hindumathy, 2011].

The overall results indicate that the crude extracts are potent antimicrobial preparations at least *in vitro*. However, *in vivo* evaluation may be required to ascertain that active concentrations of the extract when absorbed may remain bioactive for the time to completely kill the microorganisms. Further phytochemical and pharmacological studies are challenging task in order to better understand the effects of this important pharmaceutical resources. Plants of the *Hagenia* genus (Asteraceae) may prove to be a rich source of compounds with potential antimicrobial activities.

The earthworm *Pheretima posthuma* (Annelida, Megascolecidae) were used for evaluating the antihelmentic activity of the female flowers of *Hagenia abyssinica* extracts using a reference substance for comparison. The antihelmentic activity was evaluated on the earthworm *Pheretima posthuma* (Annelida, Megascolecidae) collected due to its anatomical and physiological resemblance with the intestinal round worm parasites of human. The method of (Mathew *et al*. 2012) was followed for antihelmentic screening. Emulsions of the female flowers of *Hagenia abyssinica* and the fruit of *Embelia schimperi* extracts were prepared, and further diluted to give doses of (25, 50, 100 & 200 mg/ml) emulsions.

Table 2: Antihelmintic activity of *Hagenia abyssinica* female flowers extract

Type of Extract	Dose (mg/ml)	Time (min) taken for Paralysis of Earth worms (mean values)	Time (min) taken for Death of Earth worms (mean values)
Ethanol	25	32	44
	50	24	35
	100	13	25
	200	6	18
Methanol	25	36	51
	50	29	39
	100	11	26
	200	0. 6	22
Petroleum ether	25	45	60
	50	36	50
	100	24	42
	200	16	30
n-hexane	25	46	64
	50	35	48
	100	21	32
	200	15	27
Albendazole	25	12.43±0.22	22.13±0.28
	50	9.25±0.16	18.93±0.58
	100	6±0.21	13.83±0.47
	200	-	-
Control (1% CMC Normal saline)		-	-

Albendazole solutions of the same concentrations were prepared using distilled water, and used as reference standard. The emulsion solution was diluted to 10 ml each using physiological solution, and further poured into Petri dishes. The anihelmentic activity was determined in duplicate. Four worms of about the same size per Petri dish were used. The death and/or total paralysis time were recorded at room temperature. The death of the worm was ascertained by transferring it into a beaker containing hot water at 50ºC, which stimulated and induced movements if the worm was alive. Five independent experiments were carried out for each observation to confirm the results.

Conclusions

The methanolic extracts of alcoholic extracts of *Hagenia abyssinica* female flowers have shown good antihelmentic activity and it is comparable with the effect produced by the reference drug used. The non-polar solvents extract of *Hagenia abyssinica* female flowers has shown activity which is less than the reference drug albendazole.

Conflict of interest statement

We declare that we have no conflict of interest.

Salutation

Acknowledgment

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References

1. Abegaz, M. B., Ngadjui, T. B., Bezabih, M., Mdee, K. L. 1999. Novel natural products from marketed plants of Eastern and southern Africa, *Pure Appl. Chem*.,71:919-926.
2. Aljadi, M. A., Yusoff, M. K. 2003. Isolation and Identification of Phenolic Acids in Malaysian Honey with Antibacterial Properties. Turk J Med Sci.,33:229-236.
3. Assefa, B., Glatzel, G., Buchmann, C., 2010. Ethnomedicinal uses of *Hagenia abyssinica* (Bruce) J.F Gmel. among rural communities of Ethiopia. *Journal of Ethnobiology and Ethnomedicine*. 6:20.
4. Ayo RG, Amupitan JO, and Yimin Zhao 2007. Cytotoxicity and antimicrobial studies of 1,6,8-trihydroxy-3-methyl-anthraquinone (emodin) isolated from the leaves of *Cassia nigricans* Vahl. *African Journal of Biotechnology*,6:1276-1279.
5. Ayoola, G.A., Coker, H.A., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., Ezennia E.C. and Atangbayila, T.O. 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*,7:1019-1024.
6. Caccioni, D. L. R.; Guzzardi, D.M.; Renda A. and Roberto, G. 2000. Relationships between volatile components of citrus Fruit Essential oil and Antimicrobial action on *Pencillium digitatum* and *Penicillium italicum*. *Inter Journal of Food Microbial*,88:770-75.
7. Casley-Smith J. R. 1997. Coumarin in the treatment of lymphoedema and other high-protein oedemas. *In* R. O'Kennedy and R. D. Thornes (Ed.) Coumarins: biology, applications and mode of action (p. 348). New York: John Wiley and Sons, Inc.
8. Duraipandiyan, V.; Ayyanar M. and Igancimuthu, S. 2006. Antimicrobial activity of some Ethno, edicinal plants used by paliyar Tribe from Tamil Nadu, India. *BMC Complement Altern Med*.,6:35.
9. Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. 2005. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*,4:685-688.
10. Egwaikhide, P.A. and Gimba C.E. 2007. Analysis of the phytochemical content and anti-microbial activity of Plectranthus glandulosis whole plant. *Middle-East Journal of Scientific Research:*2:135-138.
11. Eka, O.U. 1998. *Roots and Tuber Crops in Nutritional Quality of Plant Foods*. Osagie A. Eka O.A.(Eds) Post harvest Res. Unit Publication, University of Benin. pp. 1-31.
12. Evans' W.C. 1996. *Trease and Evans Pharmacognosy, 14th edition*, WB Sauders company Ltd; London. pp. 224–228, 29–309, 542–575.
13. García-Sosa K, Villarreal-Alvarez N, Lübben P and Peña-Rodríguez LM 2006. Chrysophanol, an Antimicrobial Anthraquinone from the Root Extract of *Colubrina greggii. Journal of Mexican Chemical Society*,50:76-78.
14. Gershenzon J and Dudareva N. 2007. The function of terpene natural products in the natural world. *Nature Chemical Biology*,3:408–414.
15. Harbone, J.B.1998. Phytochemical methods. *A guide to modern techniques of plant analysis, 3rdEdition*, Chapman and Hall Int. Ed, New York.
16. Heldberg I. and Stamgard F. 1989. Traditional medicine in Botswana. Ipeleng publishers, Gaborone. 324.
17. Hindumathy, C. K. 2011. *In vitro* study of Antibacterial Activity of *Cymbopogon citratus*. *World Academy of science, Engineering and Technology*,14:193-197.
18. Ibezim, E.C. 2005. Microbial resistance to antibiotics. *African Journal of Biotechnology*, 4:1606-1611.
19. Jacob, R.A. and Burri, B.J. 1996. *American Journal of Clinical Nutrition*,63:9855-9858.
20. Khan, A.M., Qureshi, R.A., Faizan, U., Gilani, S.A., Nosheen, A., Sumaira, S., Laghari, M.K. and Laghari M.Y. 2011. Phytochemical analysis of selected medicinal plants of Mar galla Hills and surroundings. *Journal of Medicinal Plants Research*,5:6017-6023.
21. Korkina LG, and Afanas'ev IB. 1997. Antioxidant and chelating properties of flavonoids. Adv Pharmacol.;38:151-163.
22. Kraft, K. And Hobbs, C. 2004. *Poket Guide to Herbal Medicine*. New York: Thieme Stuttgart,61-62.
23. Mathew Adamu, Vinasan Naidoo and Jacobus N Eloff., 2012. Some southern African plant species used to treat helminth infections in ethnoveterinary medicine have excellent antifungal activities. BMC Complementary and Alternative Medicine, 12:213
24. Mothana, R. A.; AI-Rehaily A. J. and Schultze, W. 2010. Chemical analysis and biological activity of the essential oils of two endemic *Soqotri commiphora* species. *Molecules*,15:689-698.
25. Njoku, O.V. and Obi, C. 2009. Phytochemical constituents of some selected medicinal plants. *African Journal of Pure and Applied Chemistry*:3:228-233.
26. Ojo, O. and I. Anibijuwon, 2010. Studies on extracts of three Medicinal plants of south-western Nigeria: Hoslundia opposite, lantana Camara and cymbopogon citratus. Advances in Natural and Applied Sciences,4:93-98.
27. Oloyed, O.I. 2005. Chemical profile of unripe pulp of *Carica pagaya*. Pakistan *Journal of Nutrition*,4:379-381.
28. Rhoades, David F. 1979. Evolution of Plant Chemical Defense against Herbivores. In Rosenthal, Gerald A., and Janzen, Daniel H. Herbivores: Their Interaction with Secondary Plant Metabolites. New York; Academic Press. p. 41.
29. Sengul, M., Yildiz, H., Gungor, N., Cetin, B., Eser, Z., Ercisli, S. 2009. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pak. J. Pharm. Sci.22:102-106.
30. Singh IP, Bharate SB. 2006. Phloroglucinol compounds of natural origin. Nat Prod Rep;23:558–591.
31. Sodipo, O.A., Akiniyi, J.A. and Ogunbanosu.2000. *Global Journal of Pure and Applied Scienes*,6:83-87.
32. Sofowora, A. 1993. *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Ltd., Ibadan. p. 289.
33. Wagner, H., Bladt, S. and Zgainski, E.M.1984. *Plant Drug Analysis*. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo.
34. Winter WP, K TMason, and Ford TD. 1993. Mechanism of saponininduced red cell hemolysis: reexamination; Blood 82: Suppl. 1:461.
35. Woldemariam TZ, Fell AF, Linley PA, Bibby MC, Phillips RM. 1992. Evaluation of the anti-tumor action

and acute toxicity of kosins from *Hagenia abyssinica*. J Pharm Biomed Anal.;10:555–560.

36. Yamunadevi M, Wesely EG, Johnson M. 2011. Phytochemical studies on the terpenoids of medicinally important plant Aerva lanata L. using HPTLC. A. Pacific J. of Trop. Biomedicine. S220-S225.
37. Zhang, S., Bi, H. and Liu, C. 2007. Extraction of bio-active components from Rhodiola sacha linensis under ultrahigh hydrostatic pressure. *Separation and Purification Technology*,57:275−280.