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Antimicrobial and phytochemical screening of *Euphorbia hirta* used by Abagusii Mothers in treatment of infectious diseases among young children

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Abstract
Antimicrobial and Phytochemical screening of *Euphorbia hirta* reputed to be widely used in the treatment of infectious diseases was carried out. Screening covered mainly the root, stem, leaf and flower extracts of ether. The whole plant contains compounds such as alkaloids, anthocyanins, anthracene glycosides, carotenoids, coumarins, emodins, flavonoids, saponins, steroids/triterpenoids, tannins and volatile oils, all known to be of medicinal value. The root, stem, leaf and flower extracts of ether, methanol and water inhibited the growth of all the micro-organisms that they were treated against. The most sensitive organism to these extracts was *Candida albicans* ($f_{7, 77}$=1 7.7, $p<0.001$), a stubborn fungal pathogen and the leaf and root were the most inhibitory plant extracts respectively ($F_{3, 77}$=5.9, $p<0.001$). *C. albicans* and *P. vulgaris* were the most and least sensitive microorganisms respectively $F_{7, 77}$=2.7**,** $p<0.001$). Individual plant parts did not show any significance with respect to the extraction solvent ($F_{2, 3}$=3.2, $p<0.001$). Methanol was the most efficient when the effectiveness of the extraction of solvents are compared. The result is statistically significant at (F_2, 3=3.2, $p<0.01$). Thus, the medicinal herb as used by Abagusii mothers since time immemorial has a healing agent in the treatment of oral thrush, diarrhoea and dysentery in young children.

Keywords: ether, screening, bioassay, extract, anthocyanins, diarrhoea

1. Introduction

Plants have served as the basis of sophisticated medicine systems for thousands of countries such as China and India [2]. The plant based systems continue to play an essential role in Primary Health Care (PHC). Plant products also play an important role in the health of the remaining 20% of the population who reside in both developed and developing countries. More than 80% of the population in developing countries lack access to essential medicines [17, 23]. Traditional medicine continues to play an important role in health care. In Kenya use of herbal medicine and the so-called complementary and alternative therapies is increasing dramatically. There is no single determinant of popularity but cultural acceptability of traditional practices along with perceptions of affordability; safety and questioning of the approaches of allopathic medicine all play a role.

Traditional societies in Africa and elsewhere have always relied on plants for their foods and other necessities, including their medicines [5]. Such societies had devised methods of providing everybody in the community with essential health care through acceptable, affordable and accessible means by the application of indigenous resources such as plants, animals, mineral products and certain other methods. Even today, inspite of high advances in modern medicine, people still attach great importance to the traditional system of medicine because it takes into account their socio-cultural backgrounds. African traditional medicine abounds in medicinal plants and the people wherever they do live rely on traditional medicine whereby the Abagusii are no exception. In addition, traditional healers who enjoy great prestige as the real practitioners of traditional n diviners and witch doctors also employ medicinal plants that are supposed to have spiritual or exorcising powers [17].

African medicinal plants constitute a rich but untapped pool of natural products with important potential uses. For example, the biological activities of the alkaloids are remarkable. Distinct fungicidal activities with relevance to crop protection have been found

[3, 4]. One of the present exciting biological activities of the alkaloids is their antimalarial activity. Extracts of some *Ancistrocladus* species used in traditional medicine have been shown to be high in *in vitro* activities against *P. falciparum* and also against chloroquine resistant strains [9]. Very recently, *Ancistrocladus* alkaloids have even become candidates as possible drugs against AIDS- one of the great medical and social challenges of our time [6].

In Kenya, a large proportion of people, both in the urban and rural areas rely on traditional medicine for their health care [17]. This is because of the many constraints limiting access to modern medicine as well as cultural and social reasons that have contributed to the persistence of traditional medicine even with the advent of modern medicine. Medicinal plants form the backbone of traditional medicine and their importance is not only limited to their direct use as therapeutic agents but also as starting materials for the synthesis of drugs and as models for pharmaceutically active compounds [7]. Diminishing economic resources due to high poverty levels and increased disease burdens have made allopathic medicine prohibitively expensive, thus positioning the harvesting of medicinal plants for personal use a less expensive alternative [6].

Plants provide some very powerful molluscidal agents and some of these alkaloids have been found to have activity against the tropical snail, *Biomphalaria glabra*, and the intermediate host of the tropical disease bilharzias (Schistosomiasis) [13,11]. Plant extracts have been implicated in wound healing. In plants, gums and related compounds aid in wound healing, by acting as protective agents that in accidental wounds [16 7] had reported *in vitro* antiplasmodial activity and chloroquine protection effects of fifty five organic and aqueous extracts of 11 plants used in malaria therapy in Kisii.

The list of the potential uses of medicinal plant extracts in the treatment of various human and animal disease conditions is undoubtedly lengthy and the multiplication of examples would not serve any useful purpose. The medicinal value of some of these plant extracts health care cannot be overemphasized.

1.1 Why interest in *Euphorbia hirta* L (Euphorbiaceae)

The medicinal herb has been in use by Abagusii mothers since time immemorial and has gained popularity as a healing agent in the treatment of oral thrush, diarrhoea and dysentery in young children and therefore has lingered on.

2. Materials and Methods

2.1 Extraction of *Euphorbia hirta L.*

In this preliminary screening of *Euphorbia hirta* L. the plant was separated into its various major parts that is the roots, stems, leaves and flowers. Each part was dried and then ground to a powder. The resulting extract was kept in dark properly stoppered and sealed glass containers with printed labels and stored in a dark, cool and dry cupboard in the research laboratory awaiting extraction. Fifty grams of the selected plant material was first extracted with the lipophilic solvent i.e. diethyl ether. After this the vegetable product was extracted using methanol (an intermediate polar solvent) and finally with water (a strong polar solvent). Three extractives were obtained: the ether extract (E), the methanol extract (M) and the water extract (W).

2.2 The ether extract. (E)

Fifty grams of each powdered plant part (roots, stems, leaves and flowers) material was placed in a sample thimble of the Soxhlet apparatus and the 500 ml extraction flask filled with 400 ml of redistilled diethyl ether. The extraction processes took 10 hours or until the extract was clear. This was then concentrated on a rotary evaporator at 50 °C and the resulting concentrated sample transferred to clean sterile sample bottles. This was further dried under vacuum over anhydrous copper sulphate to give a dry solid or paste of the extract for the bioassay.

2.3 The methanol extract (M)

Fifty grams of each powdered plant material was placed in the sample thimble of the Soxhlet apparatus and the 500 ml extraction flack filled with 400 ml of redistilled methanol (the re-distillation was done twice to obtain the purified form). The extraction procedures took 10 hours or until the extract was clear. This was then concentrated on a rotary evaporator at 50°C and the resulting concentrated sample transferred to clean sterile sample bottles. This was further dried under vacuum over anhydrous copper sulphate to give a dry solid or paste of the extract for the bioassay.

2.4 The water extract (W)

Fifty grams of the powdered plant material was placed in a 250 ml. Erlenmeyer flasks were then plugged with cotton wool to stop environmental contamination. This was shaken in the dark for 24 hours on a Jankee and Kunkel shaker set at 188 strokes per minute. The resulting aqueous extract was judged by loss of colour of the filtrate. Filtration was done through Whatman filter paper No. l and the filtrate was freeze-dried and ten used for the bioassay.

2.5 The bioassay

In many studies dealing with the effects of antimicrobial compounds on micro-organisms usually representative micro-organisms from Gram- positive and Gram- negative bacterial and fungal species are used [2, 18, 15]. This procedure was followed in this investigation. The test bacteria were inoculated in nutrient broth and the cultures incubated at 35 °C for 24 hours. *Candida albicans*, the test fungus, was inoculated in potato dextrose agar broth and incubated under the same conditions as the bacterial cultures. The disc diffusion method was used for the preliminary antimicrobial screening. A concentration of 10 mg/ml of each crude extract was used.

A 24-hour culture of the test organism was swabbed onto the surface of prepared sensitest agar and allowed to soak for five minutes. Sterile, soaked and dried sample discs were applied onto the surface of the inoculated sensitest agar, pressed firmly and allowed to diffuse slowly into the agar. Six discs impregnated with ether, methanol and water extracts, respectively for the roots, stems, leaves and flowers were placed on each plate. Each test was done in duplicate. Control discs impregnated with an equal volume of ether, methanol and water were dried and also placed on the inoculated sensitest agar. All inoculated plates were incubated at 35 °C for 24 hours. The diameter of inhibition was measured 24 hours after the growth of the organism. An average of the readings was then taken. Each reading was an average of six replicates.

2.6 Phytochemical screening

Basic phytochemical screening consists of performing simple chemical tests to detect the presence of classes of compounds such as nitrogenous compounds, acetogenins, polyketides, isoprenoids and carbohydrates that are known to be of medicinal value in a plant extract [20]. The general composition of an unknown vegetable product may be determined by means of a qualitative chemical analysis by extraction with different solvents of different polarities through successive and selective extractions, thus leading to the separation of the main classes of chemical constituents. Preliminary qualitative phytochemical screening were carried out for steroids (Salkowski test), terpenoids (Salkowski test), alkaloid (Wagner's Test), flavonoids (Alkaline reagent test, H2SO4 test and Lead acetate test), Tannins (Lead acetate test and Braymer's test), Saponins (Forthing test), Glycosides (Keller-Kiliani test), Coumains (NaOH test), carbohydrates (Molisch's , Benedict's test and Fehling's Test), proteins and amino acids (Xanthoproteic test and Ninhydrin test) following the standard protocols [5, 6].

2.7 Antimicrobial activity of plant extracts

Antimicrobial tests for the leaves stem and roots of *Euphorbia hirta* L (Euphorbiaceae) were carried out against the pure cultures of *Streptococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Streptococcus aureus, Bacillus subtilis, Enterobacter aerogenes, Proteus vulgaris* and *Candida albicans.* Bacteria were cultured overnight at 37˚C for 72 hour. Nutrient agar (20ml) was dispensed into sterile universal bottles. These were then inoculated, mixed gently and poured into sterile petridishes. After setting a number 3-cup borer (6mm) diameter was properly sterilized by flaming and used to make 3 uniform well in each petridishes. A drop of molten nutrient agar was used to seal the base of each cup. The wells were filled with 50 of the extract concentration of 100 g/ml and allow for diffuse (45minutes). The plates were incubated at 37˚C for 24 hours for bacteria. The zone of inhibition for the extract /fractions that showed antimicrobial activity was measured with antibiotic zone in mm.

3. Results

3.1 Extracts of Plant Parts

The antimicrobial activity was carried out on the stem, leaves, roots and flowers of *Euphorbia hirta* L. against eight micro-organisms; *S. faecalis, E. coli, P. areuginosa, S. aureus, B. subtilis, E. aerogenes, P. vulgaris* and *C. albicans.* As illustrated in Table 1.

Table 1: Plant Parts' Inhibitory Effects on Micro-organisms

	Extracts Of Plant Parts											
	Stem			Leaves			Roots			Flowers		
Micro- organisms	E	M	W	E	M	W	E	M	W	E	M	W
S. faecalis	7.0+0.1	8.2+ 0.2	8.1+ 0.1	7.2+ 0.2	11.1+0.1	7.2+0.2	7.2+0.2	10.1+ 0.2.	10.1+0.1	6.1+ 0.2	8.1+ 0.2	7.1+ 0.1
E. coli	6.2+0.2	8.2+0.3	6.1+0.4	7.2+0.2	9.5+0.1	7.1+0.1	6.1+0.2	9.1+ 0.1	8.3+0.2	7.2+0.1	8.1+0.3	7.1+0.1
P. areuginosa	7.1+0.3	8.1+0.2	7.1+0.1	8.1+0.1	9.2+0.2	7.1+0.1	7.2+0.3	7.1+ 0.2	7.1+0.1	7.2+0.2	6.1+0.2	7.1+0.1
S. aureus	8.1+0.3	8.3+0.1	7.2+0.2	9.1+0.2	9.3+0.2	6.2+0.3	8.2+0.1	7.2+ 0.2	8.2+0.3	6.4+0.2	10.1+02	7.1+0.1
B. subtilis	6.1+0.2	7.2+0.2	6.1+0.3	8.2+0.1	9.1+0.3	7.0+0.2	7.1+0.2	8.1+ 0.1	7.1+0.3	6.1+0.3	9.1+0.1	7.1+0.1
E. aerogenes	7.1+0.1	9.1+0.2	7.1+0.1	6.1+0.2	8.1+0.2	6.1+0.3	11.1+0.1	12.1+0.2	6.2+0.1	9.1+0.1	14.2+0.2	12.0+0.1
P. vulgaris	7.1+0.2	7.2+0.3	7.1+0.2	7.2+0.2	8.1+0.4	7.1+0.1	6.2+0.2	6.3+ 0.2	6.2+0.1	7.1+0.1	9.2+0.2	7.1+0.1
C. albicans	8.1+0.1	8.1+0.1	8.1+0.1	8.1+0.2	8.1+0.1	8.1+0.3	7.1+0.2	9.1+ 0.3	9.1+0.1	9.1+0.2	11.2+0.3	7.1+0.1

S. faecalis= Streptococcus faecalis; E. coli= Escherichia coli; P. aeruginosa= Pseudomonas aeruginosa; S. aureus= Streptococcus aureus; B. subtilis= Bacillus subtilis; E. aerogenes Enterobacter aerogenes; P. vulgaris = Proteus vulgaris; C. albicans= Candida albicans.

3.2 Statistics: Two way ANOVA with replication

Table 2: Phytochemical screening of *E. hirta* (whole plant) E=Ether extract; M=Methanol extract; W=Water extract

Alkaloids			Anthocyanins			Anthracene glycosides			Carotenoids		
E	M	W	E	M	W	E	M	W	E	M	W
++	+++	+	+	++	++	+	++	++	+	++	++

Key;
+ = Trace amounts, ++ = Abundant amounts, +++ = Highly abundant, - = Absent

Table 2 (cont'd): Phytochemical screening of *E. hirta* (whole plant) E=Ether extract; M=Methanol extract; W=Water extract

Emodin			Flavonoids			Polyoses			Starch		
E	M	W	E	M	W	E	M	W	E	M	W
++	+	+	+	+++	++	+	++	+	+	++	+

Table 2: (cont'd): Phytochemical screening of *E. hirta* (whole plant) E=Ether extract; M=Methanol extract; W=Water extract

Steroids/triterpenoids			Coumarins			Fatty acids			Polyuronoids		
E	M	W	E	M	W	E	M	W	E	M	W
+++	++	+		+	++	++	++	+	+	+	++

Table 2: (contd): Phytochemical screening of *E.hirta* (whole plant) E=Ether extract; M=Methanol extract; W=Water extrac

Compounds tested for											
Reducing compounds			Tannins			saponins			Volatile oils		
E	M	W	E	M	W	E	M	W	E	M	W
++	+	-	+	+++	+	+	++	+	+	+++	++

4. Discussion

The results summarized in Table 1 demonstrates that the leaf extracts have high inhibitory effects (F 11, 77= 3.2, $p<0.001$). Herein is the explanation as to why the leaves are preferred to other parts in the treatment of thrush, diarrhoea and dysentery in young children. It should also be noted that actually the whole plant is preferred in such treatments. All the plant parts have inhibitory effects on all the pathogenic micro-organisms that they were tested against whereas the results of the chemical analysis in Table 2 reveals that all the whole plant contains compounds such as alkaloids, anthocyanins, anthracene glycosides, carotenoids, coumarins, emodins, flavonoids, saponins, steroids/triterpenoids, tannins and volatile oils, all known to be of medicinal value as supported by [20].

The root, stem, leaf and flower extracts of ether, methanol and water inhibited the growth of all the micro-organisms that they were treated against. This justifies the use of the whole plant in traditional medicine in the treatment oral thrush, diarrhoea and dysentery. However, the leaves are mostly preferred. The results summarized in Table 1 and Table 2 support the reason why most traditional healers prefer to use the leaves most of the time. The dry powdered leaf extract of the whole plant is burned into ash, made into a tisane and administered orally for the treatment of acute diarrhea which is a bacterial disease brought about by toxins of bacteria such as *V. cholera*, *E. coli*, *Shigella* ssp., *S. aureus* and *Salmonella* spp. The results of this investigation clearly showed that all plant parts inhibited the growth of *E. coli* and *S. aureus* which are toxin producing bacteria associated with diarrhoea diseases. Acute diarrhoea is a major killer of children under seven years in this community.

It is also significant to note that the most sensitive organism to these extracts was *Candida albicans* ($f_{7,\ 77}$=1 7.7, $p<0.001$), a stubborn fungal pathogen and the leaf and root were the most inhibitory plant extracts respectively ($F_{3,\ 77}=5.9$, $p<0.001$). *C. albicans* and *P. vulgaris* were the most and least sensitive microorganisms respectively $F_{7,\ 77}=2.7$, $p<0.001$). Individual plant parts did not show any significance with respect to the extraction solvent ($F_{2,\ 3}=3.2$, $p<0.001$). Methanol seems to be the most efficient when the effectiveness of the extraction solvents are compared. The result is statistically significant at (F_2, 3=3.2, $p<0.01$).

4.1 Season ability and chemical constituents

The study also revealed that the Abagusii traditional healers took great exception to the time and even season when they do their plant collections. For instance the healers strongly recommend that *E. hirta* samples for medicinal preparations should be collected very early in the morning. This practice is supported by the work of other research workers who have shown that the active constituents of plants can vary in quantity and quality from season to season. This variation may only be slight in some cases but in others it is quite significant. For example, it has been shown that, apart from geographical variations observed in the constituents of *Piper guiniense* there is also a seasonal variation which is important when this plant is used in traditional medicine [1]. Similarly, [10] in their work found out that the biological activities of furastanol saponins from *Nicotiana tabacum* accumulate during ripening and are degraded during the germination of seeds.

The saponins were not found in any part of the plant. This also supports the idea that in traditional medicine the plant part to be collected is of paramount importance. This finding implies that the seeds must be harvested during the ripening time in order to have the desired medicinal value. These finding also underscores the importance of the morphological part of the plant to be collected. The saponins found in this plant showed hemolytic and fungicidal activity. The fact that the saponins showed fungitoxic activity during the bioassay would justify the use of this plant in the treatment of ringworm and other fungal infections.

There is also increasing evidence that the yield of some plant constituents can even vary within a span of 24 hours, this generally being due to the intercon version of compounds [20]. This variation in the concentration of active constituents can also be explained by the phenomenon of carbon dioxide fixation by PEP carboxylase. It is well known as part of Crussulacean acid metabolism (CAM) and is characteristic of desert succulents as supported by [19]. In CAM, carbon dioxide fixation occurs at night and carbon dioxide is released again in the day time for photosynthesis.

The malic acid produced by fixation is probably transported into the vacuole and the concentrations achieved would depress the cytoplasmic pH value to below 3 and when this is shipped back and decarboxylated in daylight it is likely to lead to a decrease in cytoplasmic pH values [12]. Such a decrease in pH values would also affect the concentration of the active principles in the plant hence, the reason why the season and even the time of harvesting of a medicinal plant are of crucial importance in the use of the plant in traditional medicine. Thus although the traditional healer is not aware of these scientific facts, at least he/she knows the best time when to collect his/her plant specimens for the optimum yield of the desired product. Out of the sixteen compounds screened for phytochemicals, methanol was involved in the extraction of eight compounds, which is half of the compounds screened for and water was involved in the extraction of eleven compounds. Methanol was second to water as an extraction solvent. This fact should be noted because water is the solvent commonly used as an extraction solvent in traditional medicine.

The active principles seem to come out more readily in the intermediate methanol extract and the water extract, a strong polar solvent. Most traditional remedies are prepared in the form of powders, saps, poultices, baths, decoctions in local gin and water, concoctions, infusions and teas. Decoctions and infusions in water are the most popular form of preparations. In this study it was revealed that most traditional healers prepared their medicinal plant potions as aqueous extracts. Other traditional healers prepare their medicinal portions in the form of tinctures (alcohol extracts) although this method was found not to be popular with the majority of healers.

These results serve as a justification for the traditional use of all plant parts of *E. hirta* in the preparation of traditional herbal remedies used in treating some of the ailments afflicting this community. The reason for this being that all the plant parts contain active therapeutic principles and the use of solvents such as water, local gin e.g. *busaa* alcoholic extract) in the preparation of decoctions, tisanes, concoctions and infusions for the extraction of compounds of medicinal value from medicinal plants can also be justified on these grounds. The age of the plant during harvesting is also considered. For the traditional healers, they usually collect young plants of for *E. hirta* for use in their herbal preparations.

Scientifically, by the fact that the age of the plant when harvested may determine not only the total amount of active constituents but also the relative amounts of each component. Such a traditional practice is supported by the work of [8] who have shown that *Ocimum gratissimum* L. produces most volatile oil per unit weight when young. This is because the number of oil- secreting hairs does not increase appreciably when the leaf increases in weight. This implies that such a plant should be harvested in the early stages of development. This observation is also supported by the work of [17] who observed that although the relative composition of terpenoids in the volatile oil of *O. gratissimum* does not vary with age [17]. There are instances where a marked change in volatile oil occurs. For example, the young plants of *Mentha piperita* L. yield mostly pulegone but this is replaced by menthonel and menthol in mature plants [20].

These observations are also supported by the work of [14] who found out those changes in the content of essential oils, flavonoids and santonin in the dry matter in some Solanaceae plants varied from 0% to5% depending on rainfall before anthesis and the plant age. The highest percentage involvement of essential oil was observed in the inflorescence at the time of anthesis and lowest in the over-ground mass during the time of growth. The oil's composition changed along with plant development. The percentage involvement of flavonoids such as quercetin and its derivatives also varied along with plant development. In the screening of *E. hirta* no quantitative determination of the active principles was done for each plant part individually. Most traditional healers prefer to use leaves in their herbal preparations. It would be an interesting topic for research to compare the relative amounts of compound in the various plant parts. Why should these healers mostly prefer the leaves and not the roots, stem, or flowers?

During these collections, the traditional healer took into consideration the morphological part of the plant collected. This practice is supported by the findings of other research works. For instance it has been found that although all aerial parts of *Ocimum gratissimum* L. produce oil of similar composition, the leaf is the richest (3.2%-4.1%) while the stem contain only traces of oil [8, 17]. Therefore, collection of the stem merely increases the bulk of plant material to be processed. For optimum yield of an active product it has been found that leaves should generally be collected as the flowers are beginning to open, flowers just before they are fully expanded and underground organs such as roots or rhizomes as the aerial parts begin to wither and die [20].

In support of these findings, [16,19], in their study on flavonol glycosides and other constituents from the leaves of *Ampelopsis brevipedunculata* traits while using the method of the distribution of these glycosides in various parts of the plant found that in the leaves, the glycosides containing arabinose and galactose were the main components. The ratio of monoglycosides to diglucosides was found to be higher in the ripe fruit than in the young fruit. These findings also lend support to the importance of the morphological part and the age of the plant during collection. The practice of considering the age of the plant during collection is also supported by other findings elsewhere. For example in West Africa, traditional healers usually use boiled yellow old leaves of *Carica papaya* L. to bathe children suffering from skin rashes. They specifically instruct their patients to use the dead leaves that have actually fallen off the tree rather than the green leaves. The yellow leaves are usually richer in phenolic constituents than the green leaves. Also the plant would have passed into the dying leaves certain unwanted metabolites that may be those require for medicinal purposes [17].

The collection of the plant parts also followed a certain pattern. For optimum yield of an active product it has been found that the collection of plant parts should generally be collected in the following order: leaves when the flower are beginning to open, flowers just before they are fully expanded and underground organs such as roots or rhizomes just as senescence of the aerial parts sets in [20]. Aerial parts were not collected during wet conditions. The explanation given for this was that if a collection is done under such conditions then the collected material would decompose thus leading to mould attack during storage. The barks were collected after or during a rainy season or damp weather, since they often peel off readily from the wood.

Gummy material or exudates from trees were collected during dry weather when they are less difficult to handle. During the collections the healers excluded unwanted matter, which would increase bulk and cause adulteration. For instance during the collection of gums, plant debris was eliminated while roots and other underground organs were freed from the soil by washing them with clean water or brushing. Each vegetable drug product was carefully examined for unwanted material such as discolored flowers or leaves diseased or spoiled from insect attack. During this investigation and literature surveys it was found that there was a relationship between the antimicrobial effects of the plant extracts and the phytochemistry of the plant.

The practice of collecting a plant at a particular time is in agreement with the observation made by [1] that the active principles of a plant can vary in quality and quantity from time to time and from season to season and that this variation may only be slight in some cases but in others it is quite significant. In one study they found that apart from geographical variations observed in the constituents of *Piper guiniense* there was also a seasonal variation that is important when this plant is used in traditional medicine. There is also scientific evidence that the yield of some plant constituents can vary within a span of 24 hours, this generally being due to the interconversions of compounds [20].

Thus, although the traditional healer is not aware of these scientific facts, at least he/she knows the best time when to collect his/her plant specimens for the optimum yield of the desired product. It would be an interesting topic for study to do a phytochemical analysis of plant extracts of *Euphorbia hirta* from plants of different ages because the traditional medical Practitioners consider the age of the plant during the time of collection. They usually collect young plants of *E. hirta* for use in their herbal preparations. This result is supported by the results of [8] who showed that *Ocimum gratissimum* produces most volatile oil per unit weight when young. They found out that the number of oil-secreting hairs do not increase appreciably when the leaf increases in weight. They, therefore, came to the conclusion that the age of the plant during harvesting may determine the total amount of active constituents and also the relative amounts of each component. The implications of such a conclusion are that the harvesting of such a plant should be done in the early stages of development. This observation is also in agreement with the work of [17] who observed that although the relative composition of terpenoids in the volatile oil of *O. gratissimum* does not vary with age, there are instances where a marked change in pulegone but this is replaced by menthone and menthol in mature plants [20].

4.2 Chemical composition and biological activity
Results in Table 2 reveals that *E. hirta L* has the following phytoconstituents; steroids/triterpenoids known to contain volatile oils, which are well known for the regulation of intestinal movements, preventing or controlling violent contractions and aiding the orderly flow of food through the bowel and inhibiting bacterial growth; tannins which are well known for their astringent action are also present. This is a confirmation that this plant is a useful remedy for oral thrush, diarrhoea and dysentery.

5. Conclusion
All the plant parts of *Euphorbia hirta* have inhibitory effects on the pathogenic micro-organisms that cause thrush, diarrhoea and dysentery in young children. The plant also has chemical compounds such as alkaloids, anthocyanins, anthracene glycosides, carotenoids, coumarins, emodins, flavonoids, saponins, steroids/triterpenoids, tannins and volatile oils, all known to be of medicinal value.

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7. Conflict of interest
The authors have declared that there is no conflict of interest with regard to the authorship and publication of this manuscript.

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