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Phytochemical and Antimicrobial analysis of leaves of *Bridelia micrantha, Cassytha filiformis, Euphorbia hirta* and *Securinega virosa*

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Abstract
This analysis was carried out to identify and compare the phytochemicals present in the n-hexane, methanol and ethyl acetate extracts of the leaves of *Bridelia micrantha, Cassytha filiformis, Euphorbia hirta and Securinega virosa* and their antimicrobial activities. The results showed presence of saponin, flavonoids, alkaloids, tannin and phenolics in the methanol extracts of the four plants. The ethyl acetate extracts of the plants showed presence of saponin, alkaloids, tannin, phenolics, glycosides while the n-hexane extracts showed presence of flavonoids, alkaloids and phenolics. The quantitative phytochemical analysis showed that *Securinega virosa* has the highest quantity of saponin, cardiac glycoside, alkaloids and flavonoids while *Cassytha filiformis* has the highest quantity of tannin. The antimicrobial analysis showed that *Staphylococcus aureus and E.coli* are most susceptible to the ethyl acetate extract of *Securinega virosa*. Salmonella spp. is most susceptible to the methanol extract of *Euphorbia hirta*. This maybe attributed to the presence of alkaloids and flavonoids in *Securinega virosa and Euphorbia hirta*.

Keywords: Antimicrobial analysis, *Bridelia micrantha*, *Cassytha filiformis*, *Euphorbia hirta*, *Securinega virosa*

Introduction
Plants are reliable sources for treatment of diseases in different part of the world. They are gifts of nature and have been in use since ancient times for curing limitless number of diseases. This practice has been recognised by WHO in its policy document since 1977 (Mohammed *et al*, 2015) [19]. Medicinal plant according to WHO (1991) [32], is any plant which in one or more of its organs contains substances that can be used for the therapeutic purposes or which are precursors for the synthesis of useful drugs. Plants cells fundamentally are chemical factories containing a rich supply therapeutically useful phytocompounds that have the potential of being developed into potent antimicrobial agents. (Adefuye and Ndip, 2013) [1]. These bioactive natural chemical compounds responsible for the therapeutic nature of plants are referred to as phytochemicals. Phytochemicals are broadly classified into two types: primary metabolites and secondary metabolites (Asha *et al*, 2015) [4]. Primary metabolites are metabolites required for the growth and maintenance of plant cells. They include vitamins, amino acids, lipids, carbohydrates, protein, nucleotides, etc. Secondary metabolites consists of the alkaloids, steroids, flavonoids, tannins, terpenoids, saponin, etc. They are end-products of primary metabolites and possess some valuable biological properties such as antioxidant activity, antimicrobial activity, antibacterial, antifungal, etc. (Rachana, 2017 and Asha *et al*, 2015) [27, 4]. According to WHO, about 21,000 plant species have potentials of being used as medicinal plants, where as more than 30% of the entire plant species are already in use (Khan, 2016) [15]. Different parts such as leaves, stem bark, root, etc. of the different plant species have different chemical constituents that make them active against diseases. These plants include *Annona muricata*, *Anarcardium occidentale*, *Vernonia amygadalina*, *Moringa olifera*, *Talinum triangulare*, *Bridelia micrantha* e.t.c. (Muanya, 2017) [20]. In the course of this study, we will be comparing the phytochemical and antimicrobial properties of four different plants ~*Bridelia micrantha*, *Cassytha filiformis*, *Euphorbia hirta* and *Securinega virosa*.

Bridelia micrantha also known as mitzeeri or the coastal golden-leaf is an evergreen tree belonging to the Euphorbiaceace family widely spread throughout the main land tropical Africa with the exception of a number of countries with very low annual rainfall (Douglas and Gitonga, 2016) [9]. It is about 20m tall with a dense widely spreading crown possessing large, alternate and simple leaves. It is found mostly in coastal forest, riverine forest, swamp forest, woodland and along forest margins. *Bridelia micrantha* is planted in agro forestry systems to provide shade and mulch. The wood is widely used for construction, poles, furniture and is suitable for flooring, interior trim etc.

The leaves are food for silkworm. and are also fed to cattles. The fruits are sweetish and edible (Bosch, 2012) [6]. Plants of similar genus ~Bridelia have shown numerous relevance in both traditional and modern medicine. According to Ramesh *et al*. (2001) [28], the stem bark of *Bridelia crenulata* showed presence of steroids, triterpene, flavone, phenol, saponin, quinone and anti microbial activities. The stem bark of *Bridelia ferruginea* is used locally for treatment of diarrhea, dysentery, gastro-intestinal disorder, gynecological disorders and rheumatic pains. The leaves are also used as a purgative and a vermifuge (Douglas and Gitonga, 2016) [9].

The stem bark, leaves and roots of *Bridelia micrantha* also have medicinal applications. The bark is used in the treatment of wounds and as purgative, abortifacient and aphrodisiac. Studies also showed that the barks are used in local treatment of cough, sore throat, headache, stomach-ache, diarrhea, sore eyes etc. in south Africa and Congo. The leaves are used as laxatives while the roots are used in treatment of symptoms of non-insulin dependent diabetes mellitus (Bosch, 2012; Douglas and Gitonga, 2016) [6, 9]. Phytochemical screening of *Bridelia micrantha* revealed presence of terpenoids, tannins, carotenoids and flavones. According to Akoegninou *et al*. (2006) [3], Tannins isolated from the bark of *Bridelia micrantha* showed antibacterial activities while the aqueous bark extracts showed anti-inflammatory activities, anti tumor and anti mutagenic properties. Methanolic and aqueous extracts of roots and stem bark of *Bridelia micrantha* were also shown to have strong activity against HIV -1 reverse transcriptase and integrase (Akoegninou *et al*., 2006; Douglas and Gitonga, 2016) [9].

Cassytha filiformis commonly known as love-vine is a species of obligate parasitic vine in the family Lauraceae with a pan-tropical distribution (GBIF, 2014) [11]. It is a perennial dextrosely-twining herb forming masses of stems up to 3 - 8m long. The leaves are scale-like and 1.5 - 2mm long. *Cassytha filiformis* is extremely wide spread, and apparently native across the America, Africa, Asia and the pacific. It occurs most commonly on coastal vegetations, usually on sandy dunes, but also on the margins of evergreen, gully and riverine forest, deciduous plateau and coastal woodlands, montane grasslands and swamp margins and in vegetation of sandy beaches of lake (USDA-ARS, 2014; Nelson, 2008; GBIF, 2014; Worth *et al*, 1979) [11, 22]. It also occurs on a wide range of hosts such as *Mangifera indica, Acacia nilotica, Eugenia aromatica, Azadirachta indica. Cassytha filiformis* however, can do considerable damage to host plants by extracting plant sap from the host and by covering the host with dense mat of stems. It reduces photosynthesis and endangers the host plant.

Cassytha filiformis has shown various importances in traditional medicine. In Nigeria, the stem and leaves are used in the treatment of Jaundice and in the suppression of lactation after still birth. It has also been reported to be beneficial in treatment of roadside eye sores, gonorrhea, kidney ailment (Nelson, 2008) [22]. According to ATRP (2010) and Nelson (2008) [22], *Cassytha filiformis* contains octoeine which have antiplatelet aggregation activity and small quantities of a poisonous alkaloid which are fatal in large doses. Extracts of *Cassytha filiformis* were also shown to have antibacterial action against *staphylococcus aureus, Escherichia Como, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, antifungal activity against *Candida albicans* (Adonu *et al*, 2013; Mythili, 2011) [2, 21].

Euphorbia hirta locally known as "Tawa-tawa" belongs to the plant family Euphorbiaceae and genus Euphorbia. It is a slender-stemmed, annual hairy plant with many branches from the base to top, spreading up to 40cm in height, reddish or purplish in color. Leaves are opposite, elliptic-oblong to oblong-lanceolate, acute or sub a cute, dark green above, pale beneath, 1-2.5 cm long, blotched with purple in the middle and toothed at the edge. The fruits are yellow, three-celled, hairy, keeled capsule, 1-2mm in diameter, containing three brown, four-sided, angular, wrinkled seeds. (Kumar *et al*, 2010; Perera *et al*, 2018) [17, 26].

Plants in Euphorbiaceae family such as *E. ingens, E. mey, E. tirucalli and E. triangular* are possible sources of rubber. Their phytochemical screening has also shown presence of flavonoids, triterpenoids, alkanes, amino acids and alkaloids. Studies have also shown that *Euphorbia hirta* possess antibacterial, anthelmintics, antiasthmatic, sedative, antispasmodic, antifertility, antifungal and antimalarial properties. *Euphorbia hirta* is used in the treatment of gastrointestinal disorders (diarrhea, dysentery, intestinal parasitosis, e.t.c.), bronchial and respiratory diseases (asthma, bronchitis, hay fever, e.t.c.) and in conjunctivitis. The stem sap is used in the treatment of eyelid styes and a leaf poultice is used on swelling and boils. Extracts of *Euphorbia hirta* have also been found to show anticancer activity (Kirtikar and Basu, 2003) [16].

Securinega virosa is a shrub belonging to the family of Euphorbiaceae about 4m high with numerous branches arising from the base and spirally arranged upwards. It is widely distributed throughout the region from Senegal to Southern Nigeria; and widespread across tropical Africa to India, China and Australia (Magaji *et al*, 2014; Burkill, 1988) [18, 7]. The plant has attractive foliage and white waxy berries. Its bushy nature ideally lends itself to Ornamental purposes, and it is commonly grown in Northern Nigeria as a hedge. The leaves are considered laxative (Burkill, 1988) [7].

Securinga virosa is a commonly used medicinal plant in African traditional medicine. In Eastern Nigeria, the root and leafy twig decoctions are used for the treatment of epilepsy. The decoctions of the leaf of *Securinega virosa* with some other plants is used in Northern Nigeria for the treatment of mental illness (Neuwinger, 1996; Magaji *et al*. 2014) [24, 18]. Studies have also shown that the methanol leaf extract of *Securinega virosa* possess antipsychotic and sedative potentials. Phytochemical screening by Magaji *et al*. (2014) [18] showed presence of saponins, flavonoids, alkaloids and tannins. It was also found to possess anti-dopaminergic activity.

Methodology

Sample Collection

Leaf samples of *Bridelia micrantha, Cassytha filiformis, Euphorbia hirta and Securinega virosa* were collected from Awka, Anambra State and were identified by a Taxonomist in Department of Botany, Nnamdi Azikiwe University, Awka.

Determination of The Phytochemical and Antimicrobial Content of The Plant Samples

Qualitative and Quantitative tests were carried out on the samples to determine the presence and amount of the phytochemicals in the ground samples.

Qualitative Phytochemical Analysis of The Plant Samples

Extraction of the Phytochemicals

10g each of the ground plant leaves were soaked in 100 ml of each of the three solvents – methanol, ethyl acetate and n-hexane. Each of the three solutions was shaken and the

mixtures were left to stand at room temperature for 48 hours after which they were filtered with Whatman No. 1 filter paper. The filtrates were collected and concentrated by heating on a rotary evaporator. The concentrated extracts were then used for the analysis.
Qualitative analyses were carried out using the standard methods described by Edeoga *et al.* (2005) [10] and Harborne (1998) [13] to ascertain the presence of phytochemicals such as tannins, alkaloids, flavonoids, etc. in the stem barks.

Test for Presence of Alkaloids.
The presence of alkaloids in each sample was investigated using the method described by Harborne (1998) [13].

Wagner's reagent test
Principle: Alkaloids under acidic condition and at room temperature react with iodine and potassium iodide to give brown reddish precipitate.
Reagent: Wagner's reagent (2g of iodide and 3g of potassium iodide are weighed, mixed and dissolved in 30 ml distilled water and made up to 100 ml with distilled water).

Procedure
1ml of the filtrate was added in a test tube followed by addition of 1ml of wagner's reagent. The solution was mixed properly and the colour change was observed. A reddish brown precipitate indicated presence of alkaloid.

Meyer's reagent test
Principle: Alkaloids under acidic conditions and at room temperature reacts with mercuric chloride and potassium iodide to give a cream coloration or precipitate.
Reagents: Dissolve Meyer's reagent (1.4 of mercuric chloride in 60 ml distilled water and 4.5g of potassium iodide in 20 ml distilled water. The two solutions are mixed and diluted to a 100 ml with distilled water).

Procedure
1ml of filtrate was added to a test tube followed by addition of 1ml of meyer's reagent in the test tube. The solution was mixed properly and the colour change was observed. A cream colour /precipitate indicated presence of alkaloid.

Test for the Presence of Steroids
Liebermann-Burchard's test was used to test for steroids. 1ml of each extract was treated with 0.5ml of acetic anhydride and cooled. This was later mixed with 0.5mL of chloroform and 1ml of concentrated sulphuric acid was carefully added using a pipette. There was the formation of a reddish brown ring which indicated the presence of steroids.

Test for the Presence of Flavonoid
The presence of flavonoid in each sample was investigated using the method described by Harborne (1998) [13].

Ammonium Test
4ml of filtrate was shaked with 1ml of dilute ammonia solution. The layers were allowed to separate and the yellow colour in the ammonical layer indicated the presence of flavonoids.

Aluminum Chloride Test
4ml of the filtrate was shaked with 1ml of 1% aluminum chloride solution. The layers were allowed to separate and the yellow colour in the aluminum chloride layer indicated presence of flavonoids.

Test for the Presence of Terpenoids
Salkowski's test was used to test for the presence of terpenoids. 5 ml of each extract was mixed with 2 ml of chloroform followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate was produced immediately indicating the presence of terpenoids.

Test for the Presence of Saponin
The presence of saponins in the samples was determined using Harborne (1998) [13] method.
10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for emulsion.

Test for the Presence of Tannin
The presence of tannins in the samples was determined using the method described by Harborne (1998) [13].

Ferric Chloride Test
To 3ml of the filtrate in the test tube, few drops of ferric chloride were added. A greenish black precipitate indicated the presence of Tannins.

Lead Acetate Test
A few drops of lead acetate were added to 3ml of the filtrate in a test tube. A cream precipitate appeared showing presence of Tannins.

Test for Cardiac glycosides (Keller Killani test)
5ml of the filtrate was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Quantitative Determination of The Phytochemical Constituents of The Plant Samples
Alkaloid Determination
The determination of the concentration of alkaloid in the leaf of *the* plants was carried out using the alkaline precipitation gravimetric method described by Harborne (1973) [12].
5 g of the powdered sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. The mixture was stood for four (4) hours at room temperature (25 ^{o}C). Thereafter, the mixture was filtered through Whatmann filter paper No. 42. The filtrate was concentrated by evaporation over a steam bath to ¼ of its original volume. To precipitate the alkaloid, concentrated ammonia hydroxide solution was added in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using previously weighed filter paper. After filtration, the residue on the filter paper is the alkaloid, which is dried in the oven at 80^{o}C. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed (Harborne, 1973; Edeoga *et al*, 2005) [12, 10].

$$\% \text{ weight of Alkaloid} = \frac{W_2\text{-}W_1}{\text{Weight of sample}} \text{x} \frac{100}{1}$$

Where
W_1 = weight of filter paper
W_2 = weight of filter paper + alkaloid precipitate (residue)

Flavonoid Determination
10g of the plant sample was extracted with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmann filter paper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Edeoga *et al*, 2005) [10].

$$\% \text{ Flavonoid} = \frac{W_2\text{-}W_1}{\text{Weight of sample}} \text{ x } \frac{100}{1}$$

Where
W_1= Weight of crucible
W_2= Weight of crucible + Flavonoid extract (residue)

Determination of Saponins
20g of the sample was added into a conical flask and 100cm^3 of 20% aqueous ethanol were added. The sample were heated over a hot waterbath for 4 hours with continuous sliming at about 55^{o}C. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over waterbath at about 90^{0}C. The concentrate was transferred into 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.
60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in waterbath. After evaporation, the sample were dried in oven to a constant weight. The saponin content was calculated in percentage (Edeoga *et al*, 2005) [10].

$$\% \text{ Saponin} = \frac{W_2\text{-}W_1}{\text{Weight of sample}} \text{ x } \frac{100}{1}$$

Where
W_1= Weight of filter paper
W_2= Weight of filter paper + Saponin extract (residue)

Tannin Determination
500mg of the sample was weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken for 1h in a mechanical shaker. This was filtered into a 50ml of volumetric flask and made up the mark. Then 5ml of the filtered was pipette out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1M HCl and 0.008M potassium ferrocyanide.

The absorbance was measured at 120nm within 10min

$$\% \text{ Tannin} = \frac{An}{As} \text{ x } \frac{C}{W} \text{ x } 100 \text{ x } \frac{Vf}{Vn}$$

An= Absorbance of test sample
As=Absorbance of standard solution
C= Concentration of standard solution
W= Weight of sample
Vf= Total volume of extract
Vn= Volume of extract analyzed

Phenol Determination
The quantity of phenol is determined using the spectrophotometer method. The plant sample is boiled with 50 mL of $(CH_3CH_2)_20$ for 15 minutes. 5 mL of the boiled sample is then pipette into 50 mL flask, and 10 mL of distilled water. After the addition of distilled water, 2 mL of NH_4OH solution and 5 mL of concentrated $CH_3(CH_2)_3CH_2OH$ is added to the mixture. The samples are made up to the mark and left for 30 minutes to react for color development and measured at 505nm wavelength using spectrophotometer.

Heamaglutinin Determination
2g of each of the sample were added 20 mL of 0.9% NaCl and suspension shaken vigorously for 1 minute. The supernatant were left to stand for 1 hour, the supernatants in each were collected and used as crude agglutination extract. Absorbance was read at 420nm.

Determination of Antimicrobial Activity
Antimicrobial screening tests (Nester *et al*., 2002)
The crude extracts and fractions of the four different plants were tested against 24 hours broth cultures of Escherichia coli, Staphylococcus aureus and Salmonella app.

Bacterial Susceptibility Test
Susceptibility test were performed on the crude extracts to ascertain their activity or not against Escherichia coli, Staphylococcus aureus and Salmonella spp. Higher concentrations of extracts were used (50 mg/ml, using methanol as solvent). In the test tube, 20ml nutrient agar (in a test tube) was melted at 100°C and stabilized at 45°C for about 15 minutes. About 0.1 ml inoculums were added from culture tubes to the agar in the test tube by the use of a loop. The test tube containing the agar and the inoculums was then rolled in between the palms gently to mix the inoculums thoroughly with the agar. The loop was flamed before it was used each time. The contest of the test tube was poured into a Petri dish and allowed to set. The Petri dishes were then labelled with the respective organism (inoculums) and date. By means of a 10mm cork borer, three cups were bored, well separated and equidistant from each other in the agar. The cups were labelled with three crude extracts. Each cup was filled with its corresponding extract to about three-quarters full. They were kept on a bench at room temperature for about 60 minutes (for the extracts to diffuse into the agar). The plates were then incubated aerobically at 37°C and examined for any zone of inhibition after 24 hours.

Determination of Minimum Inhibitory Concentration.
Four different concentrations of the antimicrobial agents were prepared (40, 20, 10 and 5 mg/ml) from the crude extracts, aqueous and also from the chloroform fractions of the various extracts. The working area was disinfected in phenol before the start of the work. 20ml nutrient agar was melted at 100°C and stabilized at 45°C for about 15 minutes in a test tube. About 0.1ml *Staphylococcus aureus* was added from culture tubes to the agar in the test tube by the use of a loop. The test tube containing the agar and the inoculums was then rolled in between the palms gently to mix the inoculum thoroughly with the agar. The loop was flamed before it was used each time. The content of the test tube was poured into a Petri dish (which was previous autoclaved at a pressure of 15 lb/in2 for 20 minutes) and to set. The Petri dish was then labelled with the name of the inoculum and date. By means of a 10mm sterile cork borer, four cups were bored well separated and

equidistant from each other in the agar. The cups were labelled with the four concentrations of the crude aqueous extract. Each cup was filled with its corresponding extract to about three-quarters full.

The Petri dish was quickly covered and then kept on a bench at room temperature for about 60 minutes (for the extracts to diffuse into agar).

The same procedure was followed for the different extracts and fractions, with the same organism and the other organisms. Thus, each extract was tested against each of the test organism, using chloramphenicol as the control for each organism. The plates were incubated aerobically at 37°C for 24 hours and examined for any zone of inhibition.

The reading was done against a dark background under reflected light. The diameters of the zones of growth of inhibition were measured with the help of a pair of dividers and rule from the underside of the covered plates for spots with inhibitions. The average of the diameters was taken. The actual zones were calculated by subtracting the diameter of the cups (10mm) from the total zone of growth. The zones of inhibition obtained were plotted against the log of concentrations to determine the minimum concentrations at which these extracts can inhibit the growth of the test organisms. The minimum inhibitory concentrations were obtained by determining the concentration at which the zone of inhibition was zero.

Results and Discussions

Table 1: Qualitative phytochemical analysis of the various extracts of leaves of *Bridelia micrantha*

Sample	Methanol Extract	Ethyl acetate extract	N-hexane extract
Saponin	+++	+	+++
Flavonoids	++	++	+
Tannin	+	-	+
Phenolics	++	+++	+++
Glycoside	-	-	-
Alkaloids	++	+	+
Steroids	+++	+++	++

Table 2: Qualitative phytochemical analysis of the various extracts of leaves of *Cassytha filiformis*

Sample	Methanol Extract	Ethyl acetate extract	N-hexane extract
Saponin	+++	+	++
Flavonoid	+++	++	++
Tannin	+	++	+++
Phenolics	+++	+++	+++
Glycoside	-	-	-
Alkaloids	+	+	+
Steroids	++	-	-

Table 3: Qualitative phytochemical analysis of the various extracts of leaves of *Euphorbia hirta*

Sample	Methanol Extract	Ethyl acetate extract	N-hexane extract
saponin	+	++	++
flavonoid	+	-	+
tannin	+	+	+++
phenolics	++	+++	++
glycoside	+	+	+++
alkaloids	+	+	+
steroids	++	-	++

Table 4: Qualitative phytochemical analysis of the various extracts of leaves of *Securinega virosa*

Sample	Methanol Extract	Ethyl acetate extract	N-hexane extract
Saponin	+++	+	+++
Flavonoid	+	++	+
Tannin	+++	-	-
Phenolics	+	+++	+
Glycoside	-	-	-
Alkaloids	-	-	-
Steroids	-	-	-

Key: + = low abundance, ++ = moderate abundance, +++ = high abundance and – = absent.

Preliminary Quantitative Phytochemical Analyses

Table 5: Quantitative phytochemical analysis of leaves of *Bridelia micrantha*

Parameters	Quantity
Saponin	3.74%
Flavonoid	4.34%
Tannin	8.79%
Phenol	9.44mg/l
Phytate	1.276mg/l
Cardiac glycosides	2.6%
Oxalate	0.142mg/l
Haemaglutinin	16.38mg/l
Alkaloid	3.18%

Table 6: Quantitative phytochemical analysis of leaves of *Cassytha filiformis*

Parameter	Quantity
Saponin	3.48%
Flavonoid	3.70%
Tannin	10.47%
Phenol	5.233mg/l
Phytate	1.160mg/l
Cardiac Glycosides	2.65%
Oxalate	0.132mg/l
Haemaglutinin	12.495mg/l
Alkaloid	3.18%

Table 7: Quantitative phytochemical analysis of leaves of *Euphorbia hirta.*

Parameter	Quantity
Saponin	3.28%
Flavonoid	4.04%
Tannin	9.58%
Phenol	8.59mg/l
Phytate	0.92mg/l
Cardiac glycosides	3.05%
Oxalate	0.15mg/l
Haemaglutinin	16.85mg/l
Alkaloid	4.58%

Table 8: Quantitative phytochemical analysis of leaves of *Securinega virosa.*

Parameters	Quantity
Saponin	4.58%
Flavonoid	4.91%
Tannin	1.97%
Phenol	12.82mg/l
Phytate	2.59mg/l
Cardiac glycosides	3.3%
Oxalate	0.190mg/l
Haemaglutinin	18.62mg/l
Alkaloid	5.16%

Results of Antimicrobial Analyses

Table 9: Zone of Inhibition (mm) of *Bridelia micrantha*

Bacterial Strains	Methanol	Ethyl acetate	N-hexane	Control
Staphylococcus aureus	-	3	-	9
E.coli	8	7	14	11
Salmonella spp	12	19	14	10

Table 10: Minimum Inhibitory Concentration (µg/ml) of *Bridelia micrantha*

Bacterial Strains	Methanol	Ethyl acetate	N-hexane	Control
Staphylococcus aureus	-	546	-	7.71
E.coli	77.5	434	10	7.56
Salmomenella spp	94	81	26.98	1881.64

Table 11: Zone of Inhibition (mm) of *Cassytha filiformis*

Bacterial Strains	Methanol	Ethyl acetate	N-hexane	Control
Staphylococcus aureus	15	-	-	12
E.coli	-	-	-	12
Salmonella spp	20	13	9	8

Table 12: Minimum Inhibitory Concentration (µg/ml) of *Euphorbia hirta*

Bacterial Strains	Methanol	N-hexane	Ethyl acetate	Control
Staphylococcus aureus	9.93	-	-	7.71
E.coli	-	173	624	7.56
Salmonella Spp	90.54	-	10	81.4

Table 13: Zone of Inhibition (mm) of *Securinega virosa*

Bacterial Strains	Methanol	Ethyl acetate	N-hexane	Control
Staphylococcus aureus	-	20.00	-	13
E.coli	-	19.00	-	9
Salmonella spp.	16	14	19	8

Table 14: Minimum Inhibitory Concentration (µg/ml) of *Securinega virosa*

Bacterial Strains	Methanol	Ethyl acetate	N-hexane	Control
Staphylococcus aureus	-	5.4	-	7.71
E.Coli	207.75	7.0	370.73	7.56
Salmonella spp	3546.39	8.61	624.34	81.64

Code: - not sensitive

Discussion

The result of the qualitative phytochemical analyses in tables 1-4 showed that the four plants contained saponin in their three different extracts. Flavonoids were also present in the extracts of the plants except the ethyl acetate of *Euphorbia hirta*. Tannins were present in the extracts of the plants except the ethyl acetate extract of *Bridelia micrantha*, ethyl acetate and n-hexane extracts of *Securinega virosa*. The analysis also showed the presence of phenolics in the four plants. Glycoside was present only in the extracts of *Euphorbia hirta* and absence in the other plants. Alkaloids was present in all the extracts of the plants except *Securinega virosa*. Steroids were present in all the extracts of *Bridelia micrantha*, methanol extract of *Cassytha filiformis*, methanol and N-hexane extract of *Euphorbia hirta*. Terpenoids were absent in all the extracts of the four plants.

The results of the quantitative analyses shown in tables 5-8 showed that *S. virosa* contained the highest quantity of saponins (4.58%), followed by *B. micrantha* (3.74%) and *C. filiformis (*3.48%). *E. hirta* had the lowest quantity of saponin (3.28%). Saponin has been reported to have anti-inflammatory and cardiac depressant properties (Okwu, 2004) [25]. *Securinega virosa* had the highest quantity of flavonoids (4.91%) followed by *Bridelia micrantha* (4.34%) and Euphorbia hirta (4.04%) while *Cassytha filiformis* has the lowest quantity (3.70%). Flavonoids are known to carry antioxidant properties and acts as antibiotics by disrupting functions of microorganisms. *Cassytha filiformis* contained highest quantity of tannins (10.47%) followed by *Euphorbia hirta* (9.58%) and *Bridelia micrantha* (8.79%) while *Securinega virosa* had the lowest quantity (1.97%). Tannins has been found to be potentially anti-viral, anti-bacterial and anti-parasitic agents, also inhibits pathogenic fungi and are also associated with many human physiological activities. At low concentrate, tannins show antimicrobial, cytotoxic and astringent properties. At high concentration, it can cause side effects such as stomach irritation, nausea e.t.c. (Wande and Babatunde, 2017; Haslam,1996) [14]. *Securinega virosa* has the highest quantity of cardiac glycosides (3.3%) followed by Euphorbia hirta (3.05%) and *Cassytha filiformis* (2.65%) while *Bridelia micrantha* has the lowest quantity (2.6%). Alkaloids were most present in *Securinega virosa*(5.16%) followed by *Euphorbia hirta* (4.58%) while *Bridelia micrantha* and *Cassytha filiformis* has the same percent of alkaloids (3.18%). Alkaloids are found to have antitumor, diuretic, antiviral, analgesics and anti-inflammatory properties (Dieu-Hien *et al*, 2019) [8].

The results of the Antimicrobial activities showed that Staphylococcus aureus appeared to be most sensitive to the methanol extract of *Cassytha filiformis* having MIC at 15 mm. However, it also appeared to be highly sensitive to the ethyl acetate of *Securinega virosa*. S.aureus was resistant to n-hexane extracts of *Bridelia micrantha, Cassytha filiformis and Securinega virosa. E. coli* was a bit sensitive to the methanol extract of *Bridelia micrantha* only by 8mm MIC, whereas it was not in the methanol extracts of other plants. *E.coli* was highly sensitive to the ethyl acetate extracts of *Euphorbia hirta* and *Securinega virosa* giving 17.3mm and 19mm respectively but slightly sensitive to that of *Bridelia micrantha* showing 7mm minimum inhibition concentration (MIC). *E.coli* showed high sensitivity to the n-hexane extract of *Bridelia micrantha* but slightly sensitive to *Euphorbia hirta*. It was however resistant to n-hexane extracts of *Cassytha filiformis and Securinega virosa*.

Salmonella spp showed a very high sensitivity to the methanol extract of *Euphorbia hirta*. It was also sensitive at varying degree to the methanol extracts of other plants. Salmonella spp was also sensitive to the ethyl acetate extracts of other plants except *Euphorbia hirta*. It also showed varying degree of sensitivity to the n-hexane extracts of all the plants.

The three bacteria strains showed higher number of sensitivity to the extracts of *Bridelia micrantha*. However, comparing the antimicrobial activities of the four plants, Staphylococcus aureus and E.coli are most susceptible to the ethyl acetate extract of *Securinega virosa* while Salmonella spp is most susceptible to the methanol extract of *Euphorbia hirta.* This may be attributed to the presence of alkaloids and flavonoids in *Securinega virosa and Euphorbia hirta* plants.

Conclusion

The whole results of the phytochemical and antimicrobial screening could explain the enthusiasm of traditional healers for these plants as drugs. The therapeutic effects of the plants are induced by the various chemical compounds found present. The antimicrobial properties of these plants, to some

extent, are the significant factors in their usage for wound management, age-related disease treatment and other bacterial-related disease treatment. Therefore, it is of great interest to carry out further screening of these plants extracts in order to reveal all their active ingredients by isolation and characterization of their antimicrobial constituents. This will help in further pharmacological evaluations.

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