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Preliminary phytochemical and mineral analyses of the root of *Hippocratea welwitschii*

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ABSTRACT

The root of the plant Hippocratea welwitschii has been used by the natives in Akwa Ibom State for the treatment of epilepsy. Phytochemical tests on the powdered root sample using standard methods of analysis showed that it contained saponins, alkaloids, phenols and glycosides. Elemental analysis of the root for chromium, calcium, magnesium, copper, manganese zinc, iron, lead, cadmium, cobalt, nickel and selenium showed the following values (0.0611, 0.2176, 0.7741, 0.0110, 0.3010, 0.0406, 0.0833,.0.0833, 0.0681, 0.1970, 0.1487, 0.239 and 0.162) mg/g respectively.

Key words:- Hippocrateae welwitschia, epilepsy, phytochemical and elemental analysis.

INTRODUCTION

The genus *Hippocratea* belongs to the family of plants known as Celastraceae *or staff vine or bittersweet family* [1]. The family has about 90 -100 genera and 1,300 species of vines, shrubs and small trees. The great majority of the genera are tropical with only *celastrus*(the staff vine) *Euoymus*(the spindles) and *maytenus* widespread in the temperate climates. Common names of some of it's genera include canotia (crucifixion thorn) catcha, *celastrus*, *Euonymus*, *hippocratea* etc[1] Most of the representatives of this family are shrubs and some as in *Hippocratea* are climbers by their branchlets, twisting round their supports.

Hippocratea welwitschii, in Liberia is called, "mano gie gbini", Ghana, "Adangme akladefi" and in Nigeria, Yoruba ",ijan" [2] Efik, "Nya woro urua mbombo" or Ibibio, "Nya woro urua mbombi," literally meaning, "coming out of the fattening room into the market". It is a shrub or climber of closed, primary or mature secondary forest or in thickets of secondary shrub from

Guinea to western Cameroon and widespread across Africa to Angola, Uganda and Tanganyika (Tanzania).

In Ghana, the root materials have been found to contain a sort of gutta of no recorded usage but only in small quantity(2.58 % crude and 1.22% pure)[2].

In Ivory Coast part of the plant is used to ease labour and delivery at childbirth[2].

Other members of the Celastraceae family that have been found to be of varied uses include :- i) *Elaeodendron buchanonii* whose timber is pale brown and fine textured. The tree yields a gum of unknown utility but in Kenya the plant is said to be extremely poisonous and that if children who have been eating the fruits and leaves drink milk , the result is usually death [2].

ii)***H . Africana***, a woody liana with tough wiry stem that cannot be broken with bare hands except with machete is used as binding material in roofing and to make high tensile ropes . They are not attacked by termites can and can also be used to weave baskets. In Nigeria the root is used to treat skin infections but examination has shown no activity against fungi nor Gram positive or Gram negative [1].

This study sought to determine the minerals and the phytochemicals both qualitatively and quantitatively contained in the root of this plant species got from Nkek Abak in Ukanafun Local Government Area of Akwa Ibom state, in Nigeria.

MATERIALS AND METHODS

The roots of this plant were dug up from an old farm in Ukanafun local govt. area of Akwa Ibom state, cleaned up to remove the sand particles and dried indoors in an airy corridor. The dried roots were then broken into smaller bits with a wooden mortar and Pestle after which they were blended into powder with a blender- Waring commercial blender 8011E model 38BL 41 and then used for both mineral and phytochemical analysis by standard methods of analysis as shown below.

Qualitative phytochemical analysis of the root powder of H.W.

Phytochemical analysis of the leaf was carried out using standard methods of analysis according to methods of (Evans, 1989, Evans and Treatise, 1989 and Sofowora, 1993), as shown below.

Test for tannins

To 5g of powdered root sample in a beaker was added 50ml of distilled water and boiled for 3 minutes on a hot plate and then filtered. To aliquot of the filtrate was added 10% ferric chloride solution. A blue or green colour would indicate the presence of tannins (Evans, 1989).

Test for phlobatannins

Aqueous extract(2ml) was boiled with 2ml of 1% HCl. Deposition of red precipitate would indicate presence of phlobatannins (SOFOWORA,1982).

Test for chlorogenic acid

To 2ml of the water extract of the plant material was added 2-3 drops of 10% ammonia solution. The mixture was heated over a flame and then exposed to air . A green colour would indicate the presence of chlorogenic acid (Evans,1989)

Test for anthraquinone

Chloroform was added to1.0g of the powdered sample material and shaken for 5mins and filtered. To the filtrate was added 5ml of ammonia solution and shaken properly. A bright pink colour in the upper aqueous layer would indicate the presence of anthraquinone (Evans,1989).

Test for saponins

To 5g of powdered root sample was added 95% ethanol and boiled for about 3mins and filtered.5ml of distilled water was added to the filtrate and shaken vigorously for 1min and allowed to stand for 30mins. Honey comb-like frothing would indicate the presence of saponin (SOFOWORA,1993).

Test for alkaloids

To 5g of powdered sample was added 95% methanol and allowed to stand for 3 days after which the content was filtered with whatman filter paper (No 1). 5ml of distilled water was added and heated up to 60oC and allowed to cool for about 10-15 mins after which 5ml of sodium hydroxide solution was added. The mixture was then watched for colour change to yellow after which 5ml of dilute HCl was added. Colour change from yellow to colourless, confirms the presence of alkaloids (Treatise and Evans,1989).

Test for glycosides

To 5ml of extract was added 2.5ml of dilute H_2SO_4 in a test tube and boiled for 15 minutes, cooled and neutralized with 10 % NaOH. Fehlings solution A and B was added. A brick red precipitation of reducing sugars would indicate the presence of glycosides.

Test for phenols

Equal volumes of extract and $FeCl_2$ solution were shaken together. Deep bluish-green solution was formed with phenols.

Test for flavonoids

A few drops of 1% ammonia solution were added to the aqueous extract of the powdered root sample in a test tube. A yellow coloration observed would mean it contains flavonoids.

Test for terpenoids

Aqueous extract (5ml) of the root sample was mixed with 2ml of chloroform in a test tube. 3ml of concentrated tetraoxosulphate VI acid was carefully added slowly by the side to the mixture to form a layer. An interface with a reddish brown coloration would be formed, if terpenoids are present.

Test for volatile oils

A small quantity of the sample was shaken with dilute sodium hydroxide (0.1m) and a dilute HCl. A white precipitate would be formed with volatile oils.

Test for balsams

Alcoholic ferric chloride (3 drops) was added to 4ml of extract which was then warmed. A dark green coloration would be formed if balsams are present.

Test for resins

To 4ml of extract was added equal volume of copper in acetic acid solution and shaken vigorously and allowed to separate. Dark blue coloration would indicate the presence of resins.

Quantitative phytochemical analysis of the root of h.w

The quantities of the phytochemicals present were determined using the methods of Harborne J.B., 1973 and Obadoni B.O. and Ochuko B.O,2001) as shown below:

Alkaloid Determination

3g of the sample were weighed into a 250ml beaker and 250ml 25% acetic acid in ethanol was added and covered to stand for 4hrs.This was filtered and the extract was concentrated using a water-bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration, dried and weighed.(Harborne JB,1973) .

Determination of total phenols

For the analysis of the phenolic component, the free fat sample was boiled with 100ml of ether for 30mins.10ml of the extract was pipette into a100ml flask, then 20ml of distilled water was added. 4ml of ammonium hydroxide solution and 10ml of amyl alcohol were also added .The samples were made up to mark and left to react for 40mins for colour development. The absorbance of the solution was read using a spectrophotometer at 550nm wavelengths (Harborne JB, 1973).

Saponin Determination

The samples were ground. 25g of each plant samples were dispersed in 250ml of 25% ethanol. The suspension was heated over a hot water bath for 4hrs with continuous stirring at about 60°C. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90°C. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous fraction was recovered while the ether layer was discarded. The purification process was repeated thrice.60ml of n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated in percentage (Obadoni BO, Ochuko PO,2001).

Antimicrobial sreening of ethanolic H.W. extract.

Pure test cultures of microorganisms were collected from the Nigerian Institute for Pharmaceutical Research and Development, Idu Abuja. FCT. They were confirmed and standardized as Klebsiella species, Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Candida albicans.

The sensitivity of the ethanolic extract of H.W. dissolved in Dimethyl sulphoxide(DMSO) were determined using the disc diffusion method of (Bauer et. al. 1966).The disk diameter was 7mm. Standardized inoculum(1-2 x 10^7 cfu/ml 0.5 McFarland standards) was each introduced onto surfaces of sterile plates and sterile glass spreader used to spread them evenly on each plate. Sterile paper discs previously inoculated with known concentrations of extract were carefully placed radially from the centre of the inoculated plates in triplicates. Amoxl and Di-methyl sulphoxide(each at 30 µg/g) were used as controls. The plates were then incubated in the oven at a temperature of 37^0C for 24hrs after which they were observed for their zones of inhibition, measured with ruler .The result is as shown in table 3.

RESULTS AND DISCUSSION

TABLE:1 Qualitative and quantitative phytochemical analysis of the root of H.W.

BIOACTIVE AGENT		QUANTITY(µg/g)
TANNINS	-	-
PHLOBATANNINS	-	-
CHLOROGENICACID	-	-
ANTHRAQUINONE	-	-
SAPONINS	+	1.66 x10^{-2}µg/g (1.66%)
ALKALOIDS	+	3.67 x10^{-3}µg/g
PHENOL	+	2.64 x 10^{-2} µg/g
BALSAMS	-	-
ANTHRACENES	-	-
FLAVONOIDS	-	-
RESINS	-	-
STEROLS	-	-
GLYCOSIDE	+	2.01 x 10^{-2}
TERPENOIDS	+	3.08 x 10^{-3}

+ = Present - = Absent

TABLE 2: mineral analysis of the root powder (mg/g)

METAL	ROOT
K	1.987
Na	2.324
Cr	0.061
Ca	0.218
Mg	0.774
Cu	0.011
Mn	0.301
Zn	0.041
Fe	0.083
Pb	0.068
Cd	0.197
Co	0.149
Ni	0.239
Se	0.162

TABLE 3: Microbial Susceptibility Testing

Test organisms	Zones of inhibition(mm)	MIC(µg/ml)	Amoxl	DMSO
Klebb.spp.	10	200	18	-
Staph.aureus	10	200	33.5	-
Baccilus subtilis	11	100	30.0	-
Escher. coli	7	250	19.0	-
Candida albicans	7	250	11.0	-

DMSO-dimethyl sulphoxide, Amoxl.-amoxicillin, MIC-minimum inhibitory concentration, Staph.aureus- staphylococcus aureus, Escher.coli - Escherichia coli

The phytochemical analysis results in table 1 above showed that the root of H.W. contains saponins, alkaloids, phenols and glycosides in varying amounts 1.66 $x10^{-2}$, 3.67 $x10^{-3}$, 2.64 x 10^{-2} and 2.01 x 10^{-2} µg/g respectively. These are responsible for the antimicrobial activities of the extract. The presence of saponins, phenols and alkaloids in the root sample could confer antibiotic property on the plant. This is supported by, the findings of Jacob et. al., 1996. Phenol, saponins and alkaloid in the root of a plant are responsible for it's use in the treatment of cough, dysentery, inflammations and ringworm (Frankel et al 1993, Jacob and Burri 1996 and Steinberg,1999).Saponins and alkaloids are toxic metabolites(Poorima and Ravishankar, 2009). Properties of saponins include, formation of forms in aqueous solution, hemolytic activity, cholesterol binding and bitterness . Their natural tendency to ward 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). They have also been reported to greatly enhance the effectiveness of certain vaccines. Plant saponins generally help humans to fight fungal infections, combat microbes and viruses and knock out some tumor cells, particularly lung and blood cancers(Barakat et al 1993; Poornima and Ravishankar,2009).They also bind blood cholesterol, thereby reducing heart problems but the most exciting and outstanding prospect for saponins are how they inhibit and kill cancer cells(poornima and raishankar,2009).It has also been reported that they do so without destroying normal cells on the process, as is the mode of some cancer fighting drugs(Ryam and Shattuk,1994; Poornima and Ravishankar,2009). Since cancer cells have more cholesterol type compounds on their membranes than normal cells, saponins therefore bind cholesterol and thus interfere with cell growth and division(Ryam and Shattuck,1994,Poornima and Ravishankar,2009). Some plant extracts containing some alkaloids(dihydrodioscorine) have been reported to possess a long lasting hypotension and contraction of the smooth muscle fibres in the intestine both in-vivo and in-vitro when administered to animals(Oliver-Bever,1989; Poornima and Ravishankar,2009).

Trace quantities of phenolic compounds help prevent the death of plants since phenolic compounds from plant extracts act as antimicrobial agents(Ofokansi et al.,2005).The presence of phenols in some plant species could mean that hey can act as anti-inflammatory, anti-clotting, anti-oxidant, immune enhancers and hormone modulators(Farguer,1996;Okwu and Omodamiro,2005 and Poornima and Ravishankar,2009).

The mineral composition of the root of H.W.(Table:2) are in appreciable concentrations. Minerals are known to play important metabolic and physiologic roles in the living system(Enechi and Odonwodo, 2003;Ujowundu et al.,2010).Iron, zinc selenium and manganese strengthen the immune system as antioxidants(Talwar et al.,1989 and Ujowundu et

al.,2010),while magnesium, zinc selenium are also known to prevent cardiomyopathy, muscle degeneration, growth retardation, alopecia, dermatitis, immunologic dysfunction, gonadal atrophy, impaired spermatogenesis, congenital malformations and bleeding disorders(Chaturvedi et al.,2004;Ujowundu etal.,2010)

Others like copper, molybdenum, chromium, cobalt etc though in trace amount are essential for survival of all forms of life but Nickel has often been associated with allergies(jewellery and jeans buttons)-CAOBISCO,1996.

CONCLUSION

From the results of this study, one could attribute the antimicrobial activity to the presence of phenols, alkaloids, saponins and terpenoids in the plant. Just as has been noticed in herbal drugs, synergy and polyvalence may be at play in the use of this plant for the treatment of epilepsy. While one may not be able to single out any one component as responsible for the beneficial activity the combined activity of all necessary phytochemical confer on it the attribute for which it is used for this ailment. For instance, synergy is often stated to occur when an extract of a plant gives greater or safer response than an equivalent dose of the compound considered to be the "active" one. An example of this is the much improved antispastic effect shown by cannabis extract compared with an equivalent dose of tetrahydrocannabidiol (Baker etal,2000,Williamson,2001;Houghton P.1999).However, in such an instance, it might well be that other active compounds are present. In whole organisms or tissue studies, other compounds present may be active against a range of targets, all contributing to the observed effect. In this instance it is polyvalence, rather than synergy which is occurring (Houghton Peter,1999; 2000). There may be other uses of H.W. apart from treatment of epilepsy going by the phytochemicals and minerals it contains.

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