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Antibiogram profile of *Lasianthera africana* (BEAUV) and *Heinsia crinata* (G. Taylor) against selected pathogenic microorganism

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Abstract: **The antibiogram profile of *Lasianthera africana* (BEAUV) and *Heinsia crinata* (two vegetables commonly used by the Efiks and Ibibios of South-Eastern Nigeria) against selected pathogenic microorganisms were studied. Antimicrobial susceptibility testing of the plant extracts against selected pathogenic microorganism using agar diffusion method showed that *Heinsia crininata* extract had a higher zones of inhibition when tested against *Vibrio choleriae* (20.4±0.11mm), *Salmonella typhi* (24.3±0.38mm) and *Candida albican* (20.3±0.25mm). However, a corresponding increase in zones of inhibition was observed when the extracts were combined (*Lasianthera africana* and *Heinsia crinata*) and tested against the selected pathogens. Qualitative phytochemical screening of the plant extracts revealed the presence of alkaloids, antheranoids, anthraquinones, cardiac glycosides, glucides, saponins, mucillages, polyphenols, reducing compounds and tannins in the plant extracts. Nevertheless, this study has revealed the abundant resources nature has provided for man since edible vegetables could also have curative abilities. It is thereby suggested that both *Heinsia crinata* and *Lasianthera africana* have therapeutic potentials and could be used as drugs either as single extracts or in combination.**

1. INTRODUCTION

In Nigeria and other developing countries especially in the tropical areas of the world the concept of treatment has been based on what man can afford within his limited resources. In these area of the world, man can afford ample plant resources (Omoya and Olukitibi, 2016). These resources have been utilized for food and treatment of diseases for example, the plant *Lasianthera african* (Editan) and *Heinsia crinatea* (Atama) have been used both for nutritive and therapeutic purposes for age in the South Eastern Nigeria (Ebana *et al.*, 1991).

The idea that a plant can be both nutritive and therapeutic is fascinating in the sense that man can unconsciously be treating himself of certain undiagnosed ailments as well as consciously providing nutrition for himself (Nascimento *et al*., 2000). On the other hand, the struggle between man and microbes has began since his appearance on earth. Man used the antimicrobial drugs against microbes since times immemorial. The employment and development of these drugs against microbes continued throughout civilizations until the modern era (Shraddha *et al.*, 2017). In the modern era, the strategy changed and scientists relied exclusively more and more on synthetic and semi-synthetic antibiotic (Harikrishma, 2013). Afterwards, interest in the magic drug "antibiotics" increased leading to new waves of synthetic antibiotics. Recently the global problem of dramatic development of bacterial resistance to synthetic antibiotics led researchers to consider the use of other natural products such as medicinal plants with antibiotic actions (Emad, 2011). Medicinal plants are rich source of compounds that are antimicrobial in nature. These plants are the source of new potential drugs and may contain compounds that have anti-inflammtory, healing, antidiabetic, anticancer, antioxidant as well as antimicrobial activities (Ayesha *et al.*, 2017). Certain plants though used for nutritive purpose have been known by local herbalists to have therapeutic effect on certain ailment. *Ocinmum grastrissimum* as well as *Venonia amygdalina* are examples. These have been used as flavonants vegetables and for therapeutic purpose (Etok and Ebana, 1996).

The present study was conducted to determine the effect of two vegetables commonly used in making soups in the South Eastern part of Nigeria on some microorganisms. This has become pertinent because of man's unending search for better options for treatment and microorganisms untiring activity of developing resistance to any new drug introduced into the market and its attendant problems.

2. MATERIALS AND METHODS

Medicinal vegetables

The plant *L.africana* (known as Editan by the Efiks and Ibibios) belongs to the Icacinaceae family. It is a globorous shrubs 3-12ft high with terete branches and small white flowers/umbellate head-like clusters in rainforest. Leaves are oblong – elliptic, long and caudate acuminate, shortly cuminate at base 8-15cm long 3-6cm broad.

Natives use the plant to treat skin infections by rubbing on skin lesions. The bitter extract is taken orally and is believed to have anti- bleeding properties, it is also used as enematics. The vegetable is used in soup preparation.

Heinsia crinata (Known as Atama by Efiks and Ibibios) is of the Rubiaceae family. It has slightly pubescent leaves, ellipitic lanceolate and actively acuminate at base about 5-10cm. it is a branching shrub growing mainly in the secondary forest or a small forest. It is about 8-25ft and sometimes up to 40ft high. Flowers are white, the fruits are small and green in colour. It is used by the natives to treat bacterial skin infections and the roots are believed to have anti-gonorrhoeal properties. The leaves are used in soup preparations.

Extraction of active principles

Two extraction methods were used in the study;

(a) One hundred grams of ground dry samples of the two plants were weighed and soaked in absolute alcohol (ethanol) using beakers for forty eight (48) hours to allow for maximum extraction of the components. The extracts were separated from the plant residue using sterile calico cloth made into bags as filter. The extracts were stored in sterile reagent bottles and used for the analysis. Whatmann No1 filter paper was used to filter sterilize the extract.

(b) Sohlet extraction as described by Cuilei (1982) was the second method used. The solvent dry used was absolute ethanol. The ground dry sample (50g) was placed in a sterile thimble in the soxhlet extractor which was fitted into the neck of the flask containing the solvent heated on a water bath. The vapour from the solvent reached soxhlet extractor through the side tube and condensed on passing into the condenser. The condensed solvent drops on the plant powder in the thimble and dissolves the required substances. The solution filters through the thimble into the flask bearing the extracted components in the solvent. This process continues until the solvent from the thimble is colourless in which case the extraction is complete. The process usually lasts about an hour. The extract obtained was stored in sterile reagent bottles and used for the analysis.

Antimicrobial susceptibility testing of extracts

The agar diffusion method of Kirby-Bauer 1966 and NCCLS, 1979 was used. Isosensitest agar (OXOID UK LTD) plates were freshly prepared and dried before use. Two to three discrete colonies of the test organisms were inoculated into peptone water, incubated at 37^0C for 6hrs and then used to flood the sensitivity agar plates. The excess was aseptically drained off. A pair of sterile forceps was used to transfer paper discs impregnated with varying concentrations of the extracts onto the Isosensitest agar plates and incubated overnight a 37^0C. all experiments were carried out in triplicates.

3. RESULTS

Table 1 present the usefulness of the plants to the natives. It revealed that *Lasianthera africana* leaf is locally called Editan and is used in treatment of skin infections by rubbing on skin lesions, as vegetable for preparing soup, as enematic and orally as antibleeding agent. *Heinsia crinata* (leaf) is locally known as Atama and is used to treat skin infections as anti- gonorrheal plant and as soup components.

The result of antimicrobial property of the plant extracts is presented in Table 2. It showed that *Heinsia crinata* extracts had a higher zones of inhibition when tested against Vibrio cholerae (20.4±0.11mm), *Salmonella typhi* (24.3±0.39mm), *Escherichia coli* (19.8±0.73mm), *Staphylococcus aureus* (19.3±0.08mm), *Pseudomonas aeruginosa* (24.3±0.25mm). However, *Lasianthera africana* extract showed a higher zone of inhibition when tested against *Klebsiella pneumonia*

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(18.5±0.03mm), as compared to that observed with extracts of *Heinsia crinata*. Table 3 present the result of antimicrobial activity of the combined extracts against the tested organisms. It showed that a higher zone of inhibition was observed with *Staphylococcus aureus* (29.6±0.40mm), as compared to other of its counterparts that had; *Candida albican* (25.7±0.06mm), *Salmonella typhi* (24.3±0,39mm), *Pseudomonas aeruginosa* (24.3±0.38mm), *Klebsiella pneumonia* (20.8±0.25mm), *Vibrio cholera* (20.5±0.90mm) and *Escherichia coli*(19.8±0.73mm). The result of the phytochemical components of the plant extracts is presented in Table 4. It showed that Alkaloids, Cardiac glycosides, Flavonoids, Phlobatinnins, reducing compounds and tannins was present in both extracts of *L. africana* and *H.crinata*.

TABLE 1: Usefulness of plants to the natives

Plant name and part used	Local name	Method and usage by natives
Lasianthera africana (leaf)	Editan	- In treatment of skin infections by rubbing on skin lesions and as vegetable for preparation soup - Used as enematic - Orally (extract as antibleeding agent.
Heinsia crinata (leaf)	Atama	- Used to treat skin infections - Extracts of rock used as anti-gonorrhoeal plant - Leaves are used as soup component

TABLE 2: Phytochemical components of plant extracts (*L. africana* and *H.crinata*)

Phytochemical components	E_1	E_2
Alkaloids	+	+
Anthranoids	+	-
Anthraquinones	+	-
Cardiac gylcosides	+	+
Flavonoids	+	+
Glucocides	+	-
Hydroxymethlanthraquinones	+	-
Mucillaages	+	-
Phlobatinnins	+	+
Polyhaenols	-	+
Reducing compounds	+	-
Saponins	+	+
Tannins	+	+

+ = present, - = absence E_1 = Ethanolic extract of *Lasianthera africana*

E_2 = Ethanolic extract of *Heinsia crinata*

TABLE 3: Antimicrobial property of the plant extracts

S/N	Test organism	E_1(zone size in mm)	E_2(zone size in mm)
1.	*Virbio cholera*	13.2±0.27	20.4±0.11
2.	*Salmonella typhi*	19.2±0.39	24.3±0.39
3.	*Eschericchia coli*	15.5±0.42	19.8±0.73
4.	*Staphylococcus aureus*	19.4±0.08	19.3±0.08
5.	*Klebsiella pneumonia*	18.5±0.03	10.8±0.25
6.	*Pseudomonas aeruginosa*	18.8±0.38	24.3±0.38
7.	*Candida albican*	17.1±0.09	20.3±0.25

Key: Mean of 3 zone of inhibition ± standard error, (Zone size in mm) E_1 = *Lasianthera africana*, E_2 = *Heinsia crinata*

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TABLE 4: Antimicrobial activity of the combined extract

S/N	Test organism	$E_1 + E_2$ (zone size in mm)
1.	*Virbio cholera*	20.5±0.09
2.	*Salmonella typhi*	24.3±0.39
3.	*Eschericchia coli*	19.8±0.73
4.	*Staphylococcus aureus*	29.6±0.40
5.	*Klebsiella pneumonia*	20.8±0.25
6.	*Pseudomonas aeruginosa*	24.3±0.38
7.	*Candida albican*	25.7±0.06

Key: Means of 3 zones of inhibition ± standard error

4. DISCUSSION

L. africana and *H . crinata* are edible vegetables used for making soups in the South Eastern part of Nigeria (mainly the Efiks and Ibibios). The effect of the aqueous and alcoholic extract of these plants were tested on some pathogenic organisms including the yeast *Candida albicans.* It was observed that the alcoholic extract of *L. africana* gave the highest inhibition (19.2±0.39mm) on *S. styphi* and the least (13.2±0.27mm) on *V.cholerae*, *H. crinata* extract showed higher inhibitions than that observed with *L. africana*. The greatest inhibition was observed on *S. typhi* and least was on *K. pneumonia*, while the aqueous extracts of both plants gave poor inhibition of the test organisms. The observation was not surprising as similar study by Andy *et al.*, (2008) reported a higher zone of inhibition when ethanolic extracts of *L. africana* and *L. crinata* were tested against *Eschericha coli, Salmonella typhi, Staphylococcs aureus* and *Candida albican*, as compared to that observed with the aqueous extracts. Also, similar study by Jude *et al.*, (2013) reported that crude extract and fractions of ethanolic leaf extract of *Lasianthera africana* exerted significant anti-leishmanial activity when tested against promastigotes of *Leishmania* major.

The two extracts were also combined and tested on the organisms. It was observed that the combined extracts gave larger inhibition zones than the individual extracts. The combined extracts had synergistic effect on some if the test organisms viz. *V. cholerae*, *S. typhic*, *E. coli*, *S. aureus* and *C. albican*. Traditional herbalists often combine two or more extracts together without actually knowing the potent plant extract nor the scientific basis for their activity (Sofowora, 1984). It is fascinating to note that when man is consciously feeding himself he is also providing treatment for himself unconsciously. This is in line with the finding of Ebana *et al.*, (1995).

This study has revealed the presence of different phytochemical components in the different extracts (crude aqueous and alcoholic). This could be explained by the fact that the phytochemical components have varying solubility regimes.

The antimicrobial potentials observed with the extracts against the test organisms could probably have been as a result of the present of these photochemicals in the extracts (saponins, tannin, flavonoids cardiac glycosides). Saponins have been reported by Mashesh and Satish (2008) as compounds that have the ability to form pores in membranes of microorganisms hence exerting a bacteriacidal effect. Tannins were also reported to have various physiological effects like anti-irritant, anti-secreleotyic, antimicrobial and antiparasitic effect (Varalakshmi *et al.*, 2014).

Phytotherapeutically, tannin containing plants have been used to treat non-specific diarrhea, inflammations of mouth and throat and slightly injured skins (Sharma *et al.*, 2009). Plant phenols are vital compounds used in eliminating the causes and effects of skin diseases and skin damage including wounds and burns (Sing *et al.*, 2011). Phenols and flavonoids are very important constituents because of their antimicrobial activity (Pawar and Arumugam, 2011).

The present study has confirmed that some of our plants are potential medicine and may have been combating various diseases in man without man's awareness. There is therefore a justification for the acceptance of these two plants not only for their nutritional values but also as medicinal plants.

5. CONCLUSION

The present study has revealed the abundant resources nature has provided for man since edible vegetables could also have curative abilities. It is hereby suggested that after these plants have gone through clinical tests, they could be accepted as drugs, as single extract or in combination.

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