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Phytochemical and Antimicrobial analysis of hulls and nuts of *Tetracarpidium conophorum* (Ukpa) on pathogenic organisms

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ABSTRACT

Nigeria is well known for various medicinal plants including *Tetracarpidium conophorum* (upka) that have long been used for the treatment of a handful of infectious diseases. *T. conophorum* is one of Nigeria's valuable medicinal plants found in the plant family *Euphorbiaceae*. This study evaluated the *in vitro* antimicrobial activity of the hulls and nut (edible) of *T. conophorum* on pathogenic microorganisms. Antimicrobial activities of the extracts from hull and nut of *T. conophorum* was evaluated on pathogenic species of *Staphylococcus aureus, Streptococcus pyogenes, E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans, Aspergillus* species using the agar well diffusion method. Phytochemical analysis of *T. conophorum* extracts on aqueous, ethanol and n-hexane showed the presence of alkaloids, glycosides, reduced compound sugar, carbohydrates, and protein in both hull and nut. Antimicrobial analysis conducted confirmed the anti-bacterial and antifungal potentials of *T. conophorum* plant. The inhibition zone diameter of the soaked hull of *T. conophorum* against the bacterial isolates was in the range of 10-18 mm while the IZD of the soaked nut of *T. conophorum* was in the range of 10-17 mm for the bacterial isolates. The IZD of the plant extracts against the fungal isolates was in the range of 11-13 mm. The ethanol and n-hexane extracts of the soaked nut of *T. conophorum* plant had little antimicrobial activity on the fungal isolates compared to the bacterial isolates which were more susceptible to the antimicrobial activity of the plant. However, the aqueous extracts of the hull and nut of the *T. conophorum* plant had inhibitory effect on the bacterial isolates but the aqueous extracts of the nut of *T. conophorum* plant had no inhibitory activity against the fungal organisms. Further molecular studies are required to characterize the active constituents responsible for the antimicrobial activity of *T. conophorum* plant.

Keywords: Phytochemical analysis, Antimicrobial activity, *Tetracarpidium conophorum*, Pathogenic microorganisms.

INTRODUCTION

Medicinal plants have over the years continued to attract the attention of pharmaceutical companies and the medical sector in particular – owing to the notable healing potentials of some herbal plants and other antimicrobial plant-derived compounds that have therapeutic effects [6, 13, 10]. Increasing incidence of antimicrobial resistance amongst some microbial pathogens has necessitated the need to turn the searchlight on herbal plants with putative antimicrobial activity for the discovery of novel antimicrobial compounds. The plant *Tetracarpidium conophorum* also known as conophor belongs to the family *Euphorbiaceae*; and it possess several nutritive and medical properties [9]. It is a west equatorial perennial plant often found growing wild as a climber in the forest and it is distributed in the southern part of Nigeria and West Africa [13, 3]. Locally, the plant is known with various names as *ukpa* (Igbo) and *awusa or asala* (Yoruba), *Ekporo* (Efik and Ibibios of Cross River and Akwa Ibom states), and *Kaso or ngak* (Cameroon). *T. conophorum*, like many plants in Africa and other parts of the world has been proven to have decorative, nutritive, medicinal, agricultural and industrial values over the years. *T. conophorum* plant is cultivated principally for the nuts which are consumed as snacks. Studies show that the nuts are rich in protein, carbohydrate, fat and oils, vitamins and minerals [7]. The ability of *T. conophorum* to reduce cholesterol seem to be the heart of their health benefits, though walnuts contain a host of other antioxidants, which helps to supports the immune system and they also possess some anticancer properties. According to [7], a cardio protective dietary fat profile is recommended for the treatment of type 2 diabetes. Decoctions and infusions made from the leaves and green fruit have been used to treat infections such as candidiasis, vaginitis, conjuctivities, glomerulo nephritis, cellulitis, endocarditis and other related diseases. Walnut extracts which are rich in dietary omega-3 fatty acids play a role in the prevention of some disorders including depression as well as dementia especially Alzheimer's disease and the antimicrobial efficacy of the plant have been attributed to its phytochemical constituents [14, 4, 3, 4]. Aside their notable antimicrobial activity, *T. conophorum* seeds have been processed and used in livestock feed formulation. A biscuit-like snack food from *T. conophorum* nut has been developed in the past, and this throws some light on the functional significance of the oilseed of the plant [1]. The oil from the nut has found use in the formulation of wood varnish, stand oil, vulcanized oil for rubber and leather substitute. Thus, this study evaluated presumptively the physicochemical constituents and *in vitro* antimicrobial activity of the hulls and nut (edible) of *T. conophorum*.

MATERIALS AND METHODS

Plant collection

The hull and nut of *Tetracarpidium conophorum* were collected in a fresh basket from a local market in Orgbu, Aniocha Government Area, Anambra State, Nigeria. The plant was identified by Dr. Mbakwe (a taxonomist) of the Department of Botany, Nnamdi Azikiwe University Awka, Anambra State, Nigeria.

Plant preparation

The hull was separated from the nut, crushed with a sterile grater and air-dried. The dried hull and nut were grinded into finer particles with the aid of a sterile mechanical grinder; and these were air-dried on a clean surface under a shade to avoid the direct effect of sunlight that could lead to photochemical reactions on the active agents of the hull and the nut. The dried and crushed hull and nut were thereafter neatly packaged and kept for the extraction process.

Plant Extraction

Aqueous, ethanol and n-hexane extraction were done on both the nut and the hull as was previously described [6, 12, 11, 3, 13]. Extraction was carried out using 20 g each of the nut and hull of *T. conophorum*. This was soaked in 200 ml of distilled water each in a conical flask and kept for 72 h in the refrigerator and in water bath at 50^{o}C for cold water extraction and for hot extraction respectively. The ethanol and n-hexane extraction was done in airtight containers to avoid the evaporation of the solvent for about 48 hrs. The extracts were obtained by filtering the contents of the flask through a Whatman No.1 filter paper. The liquid extracts were kept in a warm air oven at 50^{o}C for the solvent to evaporate.

Test organisms

The test organisms used for this study included pathogenic Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans, and Aspergillus spp. These isolates were recovered from the culture collection unit of two private laboratories (Peace Medical Laboratories and Glanson Medical Laboratories) in Awka, Anambra State, Nigeria.

Re-identification of the organisms

All the test organisms were aseptically grown on 5 ml nutrient broth overnight at 37^{o}C and then subcultured onto MacConkey agar, nutrient agar and cystein lactose electrolyte deficient (CLED) medium plates to get pure cultures of the organisms. These plates were incubated at 37^{o}C for 24 hours. The *Aspergillus* spp was cultured on Sabouraud's Dextrose Agar (SDA) plate and incubated at room temperature for 48 hours. Pure cultures of these isolates were identified biochemically using standard microbiological conventional identification techniques [5].

Determination of the antimicrobial activity

The antimicrobial activities of *T. conophorum* were evaluated on the bacterial isolates using aqueous, ethanolic and n-hexane extracts of the plant as was previously described [6, 3, 13]. Agar well diffusion method was used to evaluate the antimicrobial activity of the plant extracts. The nut and hull extracts of *T. conophorum* were reconstituted into a concentration of 10 mg/ml. Four (4) holes or wells were bored on Mueller-Hinton agar plate and on SDA plate (for *Aspergillus* spp) using a 6 mm diameter sterile cork borer. A small portion of the plant extract (50 µl) at concentrations of 10 mg/ml, 5 mg/ml, 2.5 mg/ml and 1.25 mg/ml was filled in each of the four wells. The negative control culture plate was filled with 50 µl of the solvents used for the extraction. Inoculated plates were incubated at 37^{o}C for 24 hrs and 25-28^{o}C for 72 hours for bacteria and the fungus respectively. Zones of inhibition were recorded for each tested organism.

Phytochemical Analysis

Phytochemical analysis of *Tetracarpidium conophorum* (ukpa) extracts were carried out as described previously to identify their chemical constituents (Trease and Evans, 1983). The hull and nut of *Tetracarpidium conophorum* (ukpa) were chemically tested for the presence of substances such as protein, sugar (carbohydrates), cardiac glycosides, tannin, saponins, flavonoids, and alkaloids [10, 13].

RESULTS

This study evaluated the antimicrobial activity of Tetracarpidium conophorum on some selected pathogenic organisms including Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans, and Aspergillus spp.

The result of the phytochemical analysis is shown in Table 1. Tannins, flavonoids and alkaloids are the most occurring phytochemicals determined in the *Tetracarpidium conophorum* plant. Other phytochemicals detected in the *T. conophorum* plant include reduced sugars and proteins. The antimicrobial activity of soaked hull of *T. conophorum* plant is shown in Table 2. The inhibition zone diameter (IZD) of the ethanol and n-hexane extracts of *T. conophorum* plant on *S. aureus* ranged from 10-14 mm while the IZD for *S. pyogenes* ranged from 12-17 mm. The ethanol and n-hexane extracts of *T. conophorum* plant had a better inhibitory activity on *S. pyogenes* than the *S. aureus* isolate even though both organisms are Gram positive bacteria. The inhibitory effect of the ethanol and n-hexane extracts of *T. conophorum* plant on Gram negative bacteria including *E. coli, K. pneumoniae* and *P. aeruginosa* were in the range of 12-17 mm, 14-18 mm and 14-17 mm respectively (Table 2). The ethanol and n-hexane extracts of *T. conophorum* plant also showed inhibitory effect on the growth of the fungi, *Candida albicans* and *Aspergillus* spp. The plant was more effective against *C. albicans* than the *Aspergillus* spp.

Table 1: Phytochemical screening of the plant extracts

Extracts	Tannins	Flavonoids	Alkaloids	Cardiac glycosides	Reduced sugar	Carbohydrate	Proteins	Saponins
SHE	+ve	++	++	++	+++	++	Trace	-
SHN	+ve	+++		+++	+	++	+	-
EN	+ve	-	+++	+++	++	++	++	-
EH	+ve	++	++	+	++	++	Trace	-
NN	+ve	+++	++	+++	-	+	Trace	++
NH	+ve	-	+	++	+	+++	-	-
HWH	+ve	++	++	+++	++	++	++	-
HWN	-ve	-	++	+++	++	++	+	++
CWH	+ve	+++	+++	+++	-	+++	++	-
CWN	-ve	-	++	+++	+	++	+	+
CSNN	-ve	-	+	++	++	++	-	-
CNE	-ve	-	+	+++	+++	+++	-	-

Key

SHE - Soaked Hull in ethanol; **SHN** - Soaked Hull in N-hexane; **EN** - Ethanol Nut (Soxhlet); **EH** - Ethanol Hull (Soxhlet); **NN** - N-Hexane Nut (Soxhlet); **NH** - N-hexane Hull (Soxhlet) **HWH** - Hot Water Hull; **HWN** - Hot Water Nut; **CWH** - Cold Water Hull; **CWN** - Cold Water Nut; **CSNN** - Cooked Soaked Nut in n-hexane; **CNE** - Cooked Nut Ethanol (soxhlet).

Table 2: Inhibition zone diameter of soaked hull of *T. conophorum* (in ethanol and N-hexane)

	10 mg/ml		5 mg/ml		2.5 mg/ml		1.25 mg/ml	
Organisms	**SHE**	**SHN**	**SHE**	**SHN**	**SHE**	**SHN**	**SHE**	**SHN**
S. aureus	14	13	13	12	12	10	11	0
S. pyogenes	17	14	16	13	15	12	15	12
E. coli	17	14	15	13.5	14	17	12	12
P. aeruginosa	17	15.5	15	0	14	0	10	0
K. pneumoniae	18.5	15	16	15	16	14	15	13.5
C. albicans	11	13	08	11	15	12	12	10
Aspergillus spp	0	15	14	13	12	0	13	0

Key: SHE - Soaked Hull in Ethanol; **SHN** - Soaked Hull in N-hexane

Antimicrobial analysis conducted confirmed the anti-bacterial and antifungal potentials of *T. conophorum* plant. Table 3 shows the inhibition zone diameter (IZD) of the soaked nut of *T. conophorum* plant (ethanol and n-hexane extracts) on the test organisms. The inhibition zone diameter of the soaked nut of *T. conophorum* was in the range of 10-17 mm for the bacterial isolates and 11-13 mm for the fungal isolates including *C. albicans* and *Aspergillus* species. The ethanol and n-hexane extracts of the soaked nut of *T. conophorum* plant had little antimicrobial activity on the fungal isolates compared to the bacterial isolates which were more susceptible to the antimicrobial activity of the plant. Table 4 show the antimicrobial effect of the aqueous extracts of the hull of *T. conophorum* plant against the bacterial and fungal isolates. The antimicrobial activity of the aqueous extracts of the nut of *T. conophorum* plant against the bacterial and fungal organisms is shown in Table 5.

Table 3: Inhibition zone diameter of soaked nut of *T. conophorum* (in ethanol and N-hexane)

Organisms	10 mg/ml		5 mg/ml		2.5 mg/ml		1.25 mg/ml	
	EH	**NN**	**EH**	**NN**	**EH**	**NN**	**EH**	**NN**
S. aureus	17	0	14	0	0	0	0	0
S. pyogenes	16	14	12	13	11	11	10	10
E. coli	16	14.5	23	13	19	12	12	11
P. aeruginosa	15	14	12	13	10	12.5	9	0
K. pneumoniae	21.5	18.3	15	16.5	14.3	14.5	13	13
C. albicans	13	0	11	0	12	0	0	0
Aspergillus spp	0	0	0	0	0	0	0	0

Key: EH - Ethanol Hull (Soxhlet); **NN** - N-Hexane Nut (Soxhlet)

The hot and cold water extracts of the hull of the *T. conophorum* plant had inhibition zone diameter that ranged from 10-20 mm against the bacterial isolates while the inhibition zone diameter against the fungal isolates was in the range of 10-15 mm. The hot and cold water extracts of the nut of the *T. conophorum* plant had inhibition zone diameter that ranged from 8-18 mm against the bacterial isolates while the aqueous extracts of the nut of *T. conophorum* plant had no inhibitory activity against the fungal organisms. The hull displayed a higher antibacterial activity than the nut.

Table 4: Inhibition zone diameter of soaked hull of *Tetracarpidium conophorum* (in hot and cold water)

Organisms	10 mg/ml		5 mg/ml		2.5 mg/ml		1.25 mg/ml	
	HWH	**CWH**	**HWH**	**CWH**	**HWH**	**CWH**	**HWH**	**CWH**
S. aureus	20	14	15	13	14	12	11	10
S. pyogenes	18	15	13	09	10	08	0	0
E. coli	10	13	17	12	0	0	0	0
P. aeruginosa	20	13	15	12	14	0	0	11
K. pneumoniae	0	13.5	20	12.5	23	11.5	0	0
C. albicans	0	15	0	10	0	0	0	0
Aspergillus spp	14	0	0	0	0	0	0	0

Key: HWH - Hot Water Hull; **HWN** - Hot Water Nut; **CWH** - Cold Water Hull; **CWN** - Cold Water Nut.

Table 5: Antimicrobial activity of soaked nut of *Tetracarpidium conophorum* in hot and cold water extracts with (IZD in mm).

Organisms	10 mg/ml		5 mg/ml		2.5 mg/ml		1.25 mg/ml	
	HWN	**CWN**	**HWN**	**CWN**	**HWN**	**CWN**	**HWN**	**CWN**
S. aureus	14	18	08	17	06	16	0	08
S. pyogenes	13	17	12	0	10	0	0	0
E. coli	0	0	0	0	0	0	0	0
P. aeruginosa	14	15	10	13	0	09	11	08
K. pneumoniae	0	15	21	14	13	12	0	0
C. albicans	0	0	0	0	0	0	0	0
Aspergillus spp	0	0	0	0	0	0	0	0

Key: SHE - Soaked Hull in Ethanol; **SHN** - Soaked Hull in N-hexane; **EH** - Ethanol Hull (Soxhlet); **NN** - N-Hexane Nut (Soxhlet); **HWH** - Hot Water Hull; **HWN** - Hot Water Nut; **CWH** - Cold Water Hull; **CWN** - Cold Water Nut.

DISCUSSION

T. conophorum is one of Nigeria's valuable medicinal plants found in the plant family *Euphorbiaceae*. This study evaluated the *in vitro* antimicrobial activity of the hulls and nut (edible) of *T. conophorum* on pathogenic microorganisms including *S. aureus*, *S. pyogenes*, *E coli*, *P. aeruginosa*, *K. pneumoniae*, *C. albicans*, and *Aspergillus* spp. The phytochemical analysis of *T. conophorum* extracts using aqueous, ethanol and n-hexane solvents revealed the presence of alkaloids, saponins, tannins, glycosides, reduced compound sugar, carbohydrates, and protein in both the hull and nut component of the *T. conophorum* plant. However, the hull extracts significantly showed higher presence of all the phytochemicals components such as alkaloids while the nut extracts did not show any significant presence of alkaloids and tannin as was obtainable in the hull extracts. A reasonable percentage of flavonoids were also recorded in both the hull and nut extracts. The absence of significant presence of tannins, saponins and flavonoids in the hull and nut extracts could be due to the method of extraction used. Our report on phytochemicals present in the hull and nut extracts of the plant is smaller than that of [3] who reported higher presence of tannins and flavonoids in their study. The antimicrobial activities of the *T. conophorum* plant is attributable to its rich content of phytochemicals including tannins, flavonoids and saponins as previously reported [14, 13]. Our report on the phytochemicals present in the nut and hull extracts is akin with previous study which reveal that tannins, flavonoids and saponins are responsible for the antibacterial activity of the plant hull and nut extracts [8]. Antimicrobial analysis conducted confirmed the anti-bacterial and antifungal potentials of *T. conophorum* plant. The inhibition zone diameter of the soaked hull of *T. conophorum* against the bacterial isolates was in the range of 10-18 mm. *P. aeruginosa* isolate showed reduced susceptibility to the n-hexane extracts of the soaked hull at various concentrations. However, the ethanol and n-hexane extracts of the soaked hull plant had inhibitory activity against the *S. aureus*, *S. pyogenes*, *E. coli* and *K. pneumoniae* isolates. *C. albicans* and *Aspergillus* species were susceptible to the antimicrobial activity of the ethanol and n-hexane extracts of the soaked hull plant. The inhibition zone diameter of the soaked nut of *T. conophorum* was in the range of 10-17 mm for the bacterial isolates and 11-13 mm for the fungal isolates including *C. albicans* and *Aspergillus* species. The ethanol and n-hexane extracts of the soaked nut of *T. conophorum* plant had little antimicrobial activity on the fungal isolates compared to the bacterial isolates which were more susceptible to the antimicrobial activity of the plant. The antimicrobial activity of the ethanol and n-hexane extracts of *T. conophorum* plant obtained in this study is similar to the study of [3, 13] who reported that *T. conophorum* plant posse's antimicrobial activity against pathogenic microbes. The hot and cold water extracts of the hull of the *T. conophorum* plant had inhibition zone diameter that ranged from 10-20 mm against the bacterial isolates while the inhibition zone diameter against the fungal isolates was in the range of 10-15 mm. The hot and cold water extracts of the nut of the *T. conophorum* plant had inhibition zone diameter that ranged from 8-18 mm against the bacterial isolates while the aqueous extracts of the nut of *T. conophorum* plant had no inhibitory activity against the fungal organisms. The hull displayed a higher antibacterial activity than the nut. This may be due to the significant presence of tannins in the hull extract compared with the nut extracts which had little phytochemicals. And this may also suggest that tannins possess stronger antimicrobial potential than the saponins since they are both antibacterial phytochemical constituents as was reported previously [8]. The bacterial species displayed more susceptibilities to the hull and nut extracts of the *T. conophorum* plant more than the fungal organisms which were resistant. This study reveals that *T. conophorum* plant posse's antimicrobial activity that could be harnessed for the discovery of novel antimicrobial agents [14,15]. Several nutritional, medical, agricultural and industrial benefits of *T. conophorum* abound; and thus, further studies are required to characterize the bioactive components responsible for the antimicrobial activity of *T. conophorum* plant.

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