PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF THE LEAF EXTRACT Of *Cola hispida* BRENAN & KEAY STERCULIACEAE

Supported by tetfund TERTIARY EDUCATION TRUST FUND

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Received: September 21, 2022 **Accepted:** November 12, 2022

Abstract

Plants from the Cola genus are particularly rich in secondary metabolites. *Cola hispida* is one of the well spread species of the Cola genus used ethno-medicinally for the treatment of ear infections, stomach troubles, whopping cough, malaria, depression and anxiety. In this article, we investigated the phytochemical constituents of the leaves of *Cola hispida* and their antimicrobial activity. The powdered leaves (1kg) of *Cola hispida* were extracted exhaustively with methanol via soxhlet extraction to yield the methanol crude extract. The crude extract was fractionated with solvent of increasing polarity- n-hexane, ethyl acetate, nbutanol and methanol to give n-hexane, ethyl acetate, n-butanol and methanol fractions respectively. The crude extract was subjected to qualitative and quantitative phytochemical screening using standard procedures. The crude extract and fractions were screened for antimicrobial activities using the Agar well diffusion method. The qualitative phytochemical screening of the crude extract revealed the presence of alkaloids, flavonoids, saponins, steroids, carbohydrates, resins, reducing sugar, glycosides and phenols. The quantitative estimation of secondary metabolite of the methanolic extract showed higher level of tannins followed by flavonoids, alkanoids, phenols and the terpenoids. The crude extract and fractions showed a significant broad spectrum of antimicrobial activity against the test organisms, *Escherichia Coli, Salmonella, Staphylococcus aureus, Bacillus substilis, Candida albican, Aspergillus niger.*

Keywords: Antimicrobial studies, *cola hispida*, fractionation, phytochemicals.

Introduction

Quite a number of natural products are being employed in traditional medicine practice in different countries as alternative medicine for the treatment and management of different ailments and several of these plants provide relieve of symptoms similar to that obtained from allopathic medicine (Augustina and Ufuoma, 2013). Antibiotics are one of the great advances in medicine; however, over prescription and misuse have contributed to the emergence of resistant strains (World Health, 2020). The prevalence of antibiotic resistant microorganisms has, therefore, called for the use of medicinal plants as alternative effective means of treatment and control of microbial infections (Riffel et *al*, 2002). Hence, the reason for the significant attention received by traditional medicinal plants lately from pharmaceutical industries. *Cola hispida* belonging to the sterculiaceae family is a shrub of the evergreen forest found in North and South Nigeria and West Cameroons extending into Zaire. It extracts and infusions have been used in many countries ethnomedicinally in the treatment of malaria, anxiety, stomach troubles, sea sickness, ear infections, whooping cough and depression (Blench, 1986; Odugbemi, 2016). The ethanolic extract of the stem bark and leaves have been reported to show anti-inflammatory effects in Carrageenan induced arthritis in seven day old chicks and antimicrobial properties against strains of *E*. coli, *P. aeruginosa, S. aureas and B. substilis* (Christian et al, 2012). The fruit when injected in mice has been reported to act as a stimulant creating an ecstatic and euphoric state (Uchegbu, 2016). In this article, we present the phytochemical constituents and antimicrobial activity of the leaf extract of *Cola hispida.*

Materials and Methods

Collection, Identification and Preparation of Plant Material

Fresh *Cola hispida* leaves were collected in April, 2020 at Umueko Village in Obukpa, Nsukka Local government area, Enugu State. The leaves were identified and authenticated by Dr. A. U. Okoro of the department of Botany, University of Nigeria Nsukka. The leaves were air-dried for two weeks and ground into fine powder using an electric blender, weighed and stored in an airtight container.

Preparation of Extract and Fractions

The pulverized powdered plant material (1 Kg) was placed in the thimble in the soxhlet extractor. A large round bottom flask was filled with 2.5 L of methanol. The setting was placed on a heating mantle and the methanol was allowed to boil; the extraction process was allowed to proceed for 4 hrs. The filtrate was concentrated in vacuum at reduced temperature and pressure to obtain the methanol extract (ME). The crude extract (ME) was fractionated using vacuum liquid chromatography. The "dry packing" method was used in packing the silica into the column. The extract was gradually introduced into the column and elution commenced with n-hexane. The process was completed in 2 hrs. The process was repeated on the same packed column using ethyl acetate, n butanol and methanol in their order of increasing polarity. The fractions were concentrated using rotary evaporator at 40 ^{o}C under reduced pressure to yield the n-hexane fraction (HF), ethyl acetate fraction (EF), n-butanol fraction (BF) and methanol fractions (BF) respectively. The extract (ME) and fractions (HF, EF &

BF) were used for the phytochemical and antimicrobial studies.

Phytochemical Screening

Qualitative and quantitative screening were used to ascertain the phytochemical compositions of the leaf using standard procedures as described by Harbone (1998).

Antimicrobial Assay

Four clinical bacterial (*Escherichia Coli, Salmonella, Staphylococcus aureus and Bacillus subtillis)* and two clinical fungi (a yeast fungus- *candida albican* and a mould fungus - *Aspergillus niger*) isolates were obtained from the microbial bank of the Department of Microbiology, Faculty of Biological Sciences, University of Nigeria, Nsukka. The isolates were standardized using colony suspension method and matching the strain's suspension with 0.5 McFarland standard to give a resultant concentration of 1.5×10^8 cfu/ml. The antimicrobial property was determined using the agar well diffusion technique. From the stock solution of 100 mg/ml, serial dilutions were made to obtain 50 mg/ml, 25 mg/ml and 12.5 mg/ml. Each labeled medium plate was uniformly inoculated with a test organism by using a sterile cotton swab rolled in the suspension to streak the plate surface in a form that lawn growth can be observed. A sterile cork borer of 5 mm diameter was used to make wells on the medium. 0.1 ml of the various extract and fractions concentrations were dropped into appropriate labeled well (Onoabedje *et al*, 2019). Ciprofloxacin (10 µg/ml) was used as a reference standard for the antibacterial test while fluconazole (5 µg/ml) was used as a reference standard for the anti-fungal test. The plates were incubated at 37 ºC for 24 hours for anti-bacteria test and 27ºC for 48 hours for anti-fungi test. The inhibition zone diameters (IZD) produced by each concentration of the plant extracts was measured and recorded in millimetres (mm) (10). Mean of triplicate determination was taken in each case. The minimum inhibitory concentration (MIC) was also determined.

Results

Table 1: Phytochemical screening of the methanol crude extract of *Cola hispida*

Phytochemical	Methanol extract
Alkaloids	+++
Flavonoids	+++
Saponins	+
Steriods	+
Carbohydrate	++
Resins	++
Tannins	+++
Reducing Sugar	+++
Glycoides	++
Phenols	+++

Keys: +++ High concentration, ++ Moderate Concentration, + Low Concentration, - absent

Table 2: Quantitative Phytochemical Composition of the methanol crude extract of *Cola hispida*

Phytochemical	Amount present (mg/100 g)
Alkaloids	702.0013 ±2.99881
Flavonoids	1179.8277 ± 6.99346
Saponins	0.3921 ± 0.00729
Steriods	0.7267 ± 0.02106
Terpenoids	204.8276 ± 1.67259
Tannins	1426.4260 ± 7.43142
Glycosides	771.3619 ± 1.11656
Phenols	762.3656 ± 177.04750
Cyanide	0.3362 ± 0.05018

Results are expressed in mean ± SD (n = 3)

Table 3: Inhibition zones of *Cola hispida* leaves crude extract and fractions

Extract	Conc(mg/mL)	*E. coli*	*Sal*	*S.a*	*Bacill.*	*Candida*	*A. niger*
Methanol	**100**	**13**	**16**	**10**	**12**	**20**	**19**
	50	11	8	3	8	16	15
	25	7	-	-	-	10	9
	12.5	-	-	-	-	-	-
n-hexane	**100**	**22**	**20**	**15**	**14**	**21**	**17**
	50	18	15	10	10	14	11
	25	10	8	-	-	-	-
	12.5	-	-	-	-	-	-
Ethyl acetate	**100**	**20**	**18**	**17**	**23**	**18**	**16**
	50	15	15	13	15	13	13
	25	8	7	9	7	9	7
	12.5	-	-	-	-	-	-
n-Butanol	**100**	**12**	**10**	**15**	**13**	**17**	**19**
	50	8	7	11	10	14	15
	25	-	-	7	8	7	9
	12.5	-	-	-	-	-	-
Ciprofloxacin	**10µg/mg**	**36**	**28**	**29**	**31**	**-**	**-**
Fluconazole	**5µg/mg**	**-**	**-**	**-**	**-**	**22**	**27**

E.coli = *Escherichia. Coli* Sal = *Salmonella* S.a = *Staphylococcus aureus*

Bacillus = *Bacillus substilis* Candida = *Candida albican* A. niger = *Aspergillus niger*

Table 5: Minimum Inhibitory Concentration (MIC) of Crude Extract and Fractions

ORGANISM	ME mg/ ml	HF mg/ ml	EAF mg/ ml	BF mg/ ml	Ciprofloxacine µg/ml	Fluconaz ole µg/ml
E.coli	25	25	25	50	10	-
Salmonell	50	25	25	50	10	-
S.aureus	50	50	25	25	10	-
Bacillus substilis	50	50	25	25	10	-
C. albican	25	50	25	25	-	5
A. niger	25	50	25	25	-	5

Discussion

The preliminary phytochemical screening revealed that alkaloids, steroids, flavonoids, tannins, saponins, glycosides, reducing sugars and phenols were present in the crude extract (Table1). The quantitative estimation of secondary metabolites reveals the amount of various chemical constituents present in the plant extract (table 2). The methanolic extract of *C. hispida* leaf contained 702.0013 ± 2.99881mg/g of alkaloids, 1426.4260 ± 7.43142mg/g of tannins, 771.3619 ±1.11656mg/g of glycosides, 1179.8277 ± 6.99346mg/g of flavonoids, 204.8276 ±1.67259mg/g of terpenoids, and 762.36561±77.04750mg/g of phenols amongst others. The methanolic extract showed higher level of tannins (1426.4260 ± 7.43142mg/g) than the other secondary metabolites. This was followed by flavonoids (1179.8277 ± 6.99346mg/g) and then glycosides (771.3619 ±1.11656mg/g). It is also shown that tannins, flavonoids, glycosides, alkaloids and phenols are present in high concentrations while, terpenoids are moderately present; saponins, Steroids and cyanides are present in low concentration. From this result it could be inferred that the phytochemical constituents in the leaf of *C. hispida* are mainly phenolic compounds.

The presence of tannins in the leaves of *Cola hispida* confirms the leaves to be a good source for the treatment of wounds emanating from varicose ulcers and hemorrhoids. Plants that contain tannins are used as astringents, anti-diarrhoea, diuretics, against stomach and duodenal tumours (Saxena *et al*. 2013). Flavonoids in plants possess medicinal benefits which include antioxidant and anti inflammatory

activities (Saxena *et al*, 2012). They have the ability to scavenge hydroxyl radicals, super oxide anions and lipid peroxy radicals (Okwu, 2004), hence their antioxidant activity. The flavonoids content of the leaves of *Cola hispida* therefore support its use for protection against diseases such as cancer, inflammation and atherosclerosis (Onyeka and Nwanbekwe, 2007). The presence of alkaloids in the leaves of *Cola hispida* supports the finding that the antibacterial activity of this plant may be attributed to the alkaloids (Onyeleke *et al*. 2008). Alkaloids have been reported to possess various pharmacological activities including anti-hypertensive effects, antiarrhythmic effect, anti-malaria and anti-cancer activity (Saxena *et al*, 2013). The presence of phenols in the *Cola hispida* serves as antiseptic and reduces inflammation when taken internally; these bioactive agents have an irritant effect when applied to the skin (Persinos, 1967).

At concentration of 50 mg/ml all the test organisms were susceptible to both the crude extract and fractions. The zone of inhibition of the extract / fractions, assayed against the test organisms ranged between 3mm and 22 mm. The Ciprofloxacin (10 µg/ml) antibacterial positive control, produced zones of inhibition that ranged from 28 to 36 mm while fluconazole (5 µg/ml) antifungal positive control produced zones of inhibition that ranged from 22 to 27 mm. The MIC of *C. hispida* for methanol crude extract was 50 mg/ml for all the bacterial strains and 25mg/ml for the fungi; MIC for n-hexane fraction was 25 mg/ml for *salmonella typhi* while 50 mg/ml for *Escherichia Coli, Staphylococcus aureus, Bacillus substilis, Candida albican* and *Aspergillus niger*; MIC for ethyl acetate fraction was 25mg/ml for all test; and for n-butanol fraction MIC was 50 mg/ml for *salmonella* and 25mg/ml for *Escherichia Coli, Staphylococcus aureus, Bacillus substilis, Candida albican, Aspergillus niger* (Table 8). The variations in degree of inhibition and MIC shown by the various test organisms may be due to the nature and level of the antimicrobial agents present in the extract / fractions and their mode of action on different test microorganisms [42 17].The ethyl acetate fraction exhibited the best antimicrobial activity as it inhibited the growth of all the test organisms at the concentration of 25mg/ml and also has the highest zone of inhibition of 23mm. The least IZD (3mm) was shown by the methanol fraction against *Staphylococcus aureus* at 50mg/ml. The n-hexane showed the best antifungal property as shown from its zone of inhibition (19 – 20mm). Statistical analysis using Krustal-Walis test showed that there was no significant difference ($p > 0.05$) in the antimicrobial activities of the extract and fractions. bioactive substances such as alkaloids, saponins, tannins, flavonoids, cardiac glycosides, phenols, etc has been reported to be responsible for the inhibition of microbial growth by plant extract (43, 44 18,19). Therefore, the observed antimicrobial action of *C. hispida* can be said to be due to the presence of the phyto-constituents.

Conclusion

This research work has justified some claims of the use of *Cola hispida* in ethnomedicine. The broad spectrum of activity exhibited may make the extract attractive as an antibacterial and antifungal agent for various applications in medicine, agriculture, food preservation, cosmetics and nutraceuticals. This study undoubtedly confirms that the leaves of *Cola hispida* contain numerous phytochemicals that have potential antibacterial and antifungal effects that could be of clinical benefits.

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