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Antimicrobial Activity of the Masticatory *Cola acuminata* Nut (Gooro)

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Abstract: The aim of this study was to investigate the possible inhibitory activity of *Cola acuminata* seed (Petroleum ether, methanolic and aqueous) extracts against two standard Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*), three Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonae*) bacteria and one fungus (*Candida albicans*). Petroleum ether, methanolic and aqueous extracts of *Cola acuminata* nuts at concentrations of 10, 12.5, 25 and 50% were screened for antimicrobial activity against two standard Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*), three Gram negative (*E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoneae*) bacteria and one fungus (*Candida albicans*). No inhibitory activity was detected using all concentrations of petroleum ether extract for all tested microorganisms. Wide range of activity was exhibited by both aqueous and methanol extract. However, the former highest activity was detected against *Staphylococcus aureus* at the two concentration 25 and 50% (20 and 24 mm) while the latter was found against *Candida albicans* at the concentration of 25% (20 mm).

Key words: Antimicrobial activity, *Bacillus subtilis*, *Candida albicans*, *Cola acuminata*, *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoneae*, *Pseudomonas aeruginosa*

INTRODUCTION

The demand for safer and natural foods has been increasing since consumers have become more concerned over the presence of chemical residues in their stuff. There is however, a strong demand for the reduction or elimination of the synthetic chemical. It has long been recognized that some plant materials exhibit antimicrobial properties. The use of these plant materials as preservatives and as means of preventing microorganism development in foods has become the subject of extensive studies (Gould, 1996).

In particular, the inhibitory effects of extracts of many kinds of herbs and spices against food borne bacteria and pathogens have been reported. Among these are cassia, clove, garlic, sage and thyme (Shelef, 1983; Zaika and Kissinger, 1981; Saleem and Ai-Delaimy, 1982; Tassou *et al*., 2000).

Currently there is growing demand worldwide of consumers to minimize chemical preservation that can be detrimental to human health (Cho *et al*., 1995; Smid and Gorris, 1999). Consequently, spices, herbs and naturally occurring phenolics from various plant sources are being studied in detail in response to consumer requirements for fresher and more natural additive-free products (Nychas, 1995; Tassou *et al*., 1997).

Cola, a member of the chocolate family Sterculiaceae (from which nuts are obtained) a tree probably originally from western tropical Africa (Encyclopedia Americana, 1988). The cola nut is a capsule-shaped fruit composed of fleshy, irregularly shaped seeds. The seeds of *Cola acuminata* are pink, red or white when fresh, and become brown and hard once they are dried. Their bitter and astringent taste is why these seeds are called nuts. Chewing of the fresh *Cola acuminata* nut is a wide spread habit of the Western and sub-Saharan indigenous populations of Africa especially in Northern Nigeria, Ivory Coast, Sierralione Liberia and Sudan (Rusell, 1955; Purseglove, 1968; Rosengarten, 1984), while the dried nuts are used beverages and pharmaceutical purposes in Europe North America (Ayodele, 1995).

The *Cola acuminata* nut's primary social and economic significance lies in its being a very concentrated source of Central Nervous System (CNS) stimulant, caffeine which may help in relieving migraine (Kiple and Ornelas 2000b), Theobromine which act as cerebral vasodilator and thought to relieve pain and neuralgia (Hirt and M'pia, 2001) and proanthocyanidin is used as anti-trypanosoma compound effective against *Trypanosoma bruci* (Kubata *et al*., 2005). They also ease hunger and thirst, eliminate fatigue, provide energy by stimulating the muscles and nerves and enhance intellectual activity

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Table 1: Differential characteristics of test bacteria

Characteristics	*S. aureus*	*E. coli*	*Ps. aeruginosa*	*B. subtilis*	*Klebsiella* spp
Gram stain	+ve	-ve	-ve	+ve	-ve
Motility	+ve	+ve	+ve	+ve	-ve
Catalase	+ve	+ve	+ve	+ve	+ve
Nitrate	+ve	+ve	+ve	+ve	+ve
Arginine	+ve	+ve	+ve	+ve	-ve
Indole	-ve	+ve	-ve	-ve	-ve
Methyl red	-ve	+ve	+ve	-ve	-ve
Voges-Proskauer	+ve	-ve	-ve	+ve	+ve
Gas production from glucose	-ve	+ve	+ve	-ve	+ve
Acid production from glucose	+ve	+ve	-ve	-ve	+ve
Acid production sucrose	-ve	+ve	+ve	+ve	-ve
Acid production from lactose	+ve	+ve	-ve	+ve	+ve
Oxidase	-ve	+ve	+ve	+ve	-ve
Urease	+ve	-ve	+ve	+ve	+ve

+ve = 90% or more strains had positive reaction; -ve = 90% or more strain had negative reaction; -: Non- determined

(Sundstrom, 1966; Nickalls, 1986). Traditionally, the leaves, flowers, fruit follicles and park of *Cola acuminata* were used to prepare a tonic as a remedy for dysentery, cough, diarrhea, and vomiting (Ayensu, 1978).

MATERIALS AND METHODS

Plant materials: *Cola acuminata* seeds were purchased from a local market in Khartoum, authenticated by the scientists at the Aromatic and Medicinal Plants Research Institute, Khartoum, and brought to the research laboratory, University of El Neelain, Khartoum, Sudan. The plant tissues were cleaned, shade-dried, powdered by a mechanical grinder.

Standard microorganisms: The test organism used in this study were kindly provided by the scientists at the National Health Institute, Khartoum and designated as follows:

Bacteria:
Bacillus subtilis- NCTC/8236
Staphylococcus aureus- ATCC/25923
Escherichia coli- ATCC/27853
Klebsiella spp- ATCC/3565
Pseudomonas aeruginosa- ATCC/2785
NCTC National Collection Type Culture, London, UK.
ATCC American Type Culture Collection, Manassas, AV, USA.

Fungus:
Candida albicans

Antibiotics:
Gentamicin (Roussel Laboratories Ltd, England)
Nystatin (Sigma Chemical Company, USA)

Cultural media: Standard media, nutrient broth (Oxoid Ltd., London) form the base of most of the media used. Nutrient agar (Oxoid Ltd., London) was used to prepare the enriched culture media.

Mueller- Hinton agar (Oxoid, Ltd, London), used for all antibiotic sensitivity test as standard drug as well as for plant extracts evaluation.

Sabouraud's Dextrose agar (Difco, USA), used as enriched culture media for fungi

Preparation of crude extracts: Twenty grams of powdered *Cola acuminata* seeds were extracted successively with petroleum ether 90% at 37ºC and methanol 95% at 37ºC using Soxhlet apparatus extractor for 3 h. The extracts were evaporated under reduced pressure, air-dried and yields were recorded. The aqueous extract was dried by freeze dryer and weighed. The extracts of the seeds were reconstituted at the time of testing in concentrations of 50, 25, 12.5 and 10%.

Preparation of stock extracts solution: One grams of each extract was dissolved in 1 ml. of the same solvent used for extraction.

Preparation of the test organism: The properties at the standard bacteria are summarized in (Table 1).

***Staphylococcus aureus*:** Gram-positive, non-motile, non-spore forming, aerobic, facultatively anaerobic organism, 0.5 mm in diameter. It occurs singly, in pairs or irregular and many of the strain form a golden yellow pigment on colony of good growth on ordinary media. Catalse and urease positive, oxidase and indol negative.

***Escherichia coli*:** Gram-negative rod, 1.1-1.5 mm wide and 2.0-6.0 mm long with rounded ends and shape varying from coccoid to rod, motile, aerobic, facultatively anaerobic oxidase and urease negative, citrate can not be used as a sole carbon, most strain are fermenters of methyl red positive, catalase and indol positive and VP negative.

***Pseudomonas aeruginosa*:** Gram-negative bacillus, non-spore forming, non- capsulated, motile by one or two polar flagella aerobic, facultatively anaerobic, grow on a wide variety of culture media, catalase positive.

Table 2: Evaluation of antimicrobial activity of *Cola acuminata* seed petroleum ether, methanol and aqueous extracts

		Inhibition zone (mm)		
Organism	Extract conc. %	Petroleum ether extract	Methanol extract	Aqueous extract
Bacillus subtilis	10	(-)	14-15	11-12
	12.5	(-)	10-12	14
	25	(-)	18	14
	50	(-)	16	18
	10	(-)	12-12.5	12
Staphylococcus aureus	12.5	(-)	13	10
	25	(-)	15	22
	50	(-)	17	24
	10	(-)	11-12	10-14
Escherichia coli	12.5	(-)	9	8
	25	(-)	10	9
	50	(-)	11	11
	10	(-)	14-15	14-15
Klebsiella pneumonae	12.5	(-)	11	10
	25	(-)	12-13	9
	50	(-)	9-10	10
	10	(-)	12-14	11-12
Pseudomonas aeruginosa	12.5	(-)	11	10
	25	(-)	11-12	11
	50	(-)	18	12
	10	(-)	13-14	13-15
Candida albicans	12.5	(-)	14	14
	25	(-)	20	9
	50	(-)	18	8

(-) = No inhibition zone was observed

***Bacillus subtilis*:** *Bacillus subtilis*, known also as the hay bacillus or grass bacillus, is a Gram-positive, catalase-positive bacterium commonly found in soil. A member of the genus *Bacillus*, *B. subtilis* is rod-shaped, and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions. Unlike several other well-known species, *B. subtilis* has historically been classi fied as an obligate aerobe, though recent research has demonstrated that this is not strictly correct.

***Klebsiella pneumonae*:** Gram-negative, non-motile straight rods, 0.3-1.0×0.06-6.0 μm, arranged singly, in pairs or in short chain; often surrounded by a capsule, facultatively anaerobic, having both a respiratory and a fermentative metabolism.

Antimicrobial assay: The antimicrobial activity of the extract was assessed by the agar-well diffusion method (Kingsbury and Wagner, 1999). The nutrient agar medium was properly and separately inoculated with the standard organism at 10^6 cfu/mL to achieve confluent growth and allow drying at room temperature. On each inoculated plate, 10 mm-diameter wells were bored in the agar using a sterile cork borer. Hundred μl aliquot of each concentration (50, 25, 12.5 and 10%) was reconstituted extract and placed into the well using a standard Pasteur pipette. The agar was allowed to solidify before incubation at 37ºC for 18 h for bacteria and 25ºC for 72 h for the yeast.

The diameter of clear zones around each well was measured (mm) after incubation to determine the strength of inhibition. Three replicates were made from each concentration and comparative activity was recorded. The antimicrobial activity of the plant extract against the standard microorganism was evaluated and compared with that of Gentamicin (Bacteria) and Nystatin (Yeast).

RESULTS AND DISCUSSION

Extract yield: The 50 g of *Cola acuminata* powdered seed yielded 0.9, 4.8 and 6 g when extracted by petroleum ether, methanol and water respectively.

Evaluation of *in vitro* antimicrobial activity of petroleum ether, methanol and aqueous extracts of *Cola acuminata* seeds: The inhibitory activity of the different *Cola acuminata* extracts are presented in Table 2. Results indicated that the petroleum ether extract had no inhibitory activity against both bacteria and yeast. The methanolic and aqueous extracts of *Cola acuminata* seeds exhibited a wide range and a broad- spectrum antimicrobial activity against all the test bacteria and fungi ranging from 8-20 and 8-24 mm, respectively and this was compared well with Gentamicin and Nystatin (Table 3, Fig. 2). The highest inhibitory effect of the aqueous extract was observed against *Staphylococcus aureus* at the concentrations of 25 and 50% (Fig. 1), whereas the 25% concentration of the methanolic extract showed the highest inhibitory effect against *Candida albicans* (20 mm). The methanolic extract of *Kigelia africana* exhibited high activity than aqueous and chloroform extracts against *Salmonella typhi* but moderate activity against *Staphylococcus aureus*. Although, it was

Fig.1: Inhibition zone produced by methanol extract (10, 12.5, 25, 50%) of *Cola acuminata* seeds against *Staph. aureus*

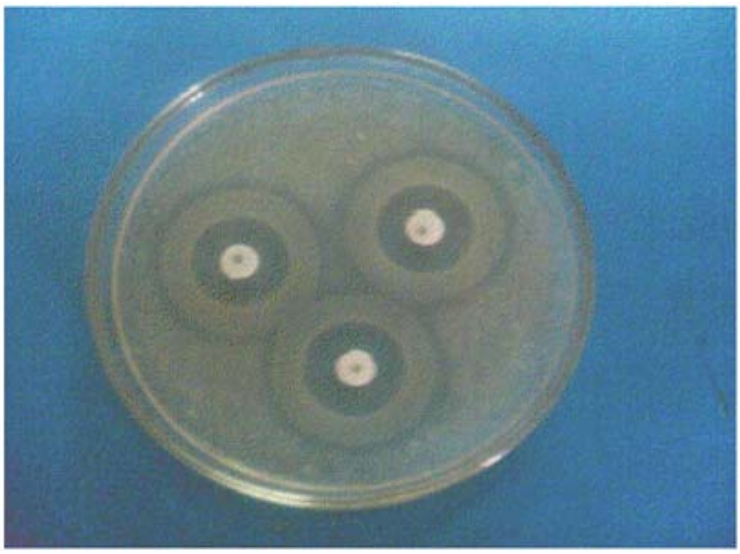

Fig. 2: Inhibition zone produced by Gentamicin against *Staph aureus*

Table 3: Evaluation of antimicrobial activity of Gentamicin and Nystatin

Gentamicin (10 mg/mL)	Inhibition zone (mm)
B. subtilis	18
S. aureus	24
E. coli	20
Kl. Pneumonae	17
Ps. aeurignosa	15
Nystatin (10 mg/mL)	
Candida albicans	14

reported that methanol was a better solvent for the consistent extraction of antimicrobial substances from medicinal plants when compared to other solvents such as aqueous, ethanol, chloroform and hexane (Lin *et al*., 1999; Ahmed *et al*., 1998; Eloff, 1998), the methanolic extract of *Parinarium glaberrimum* Hassk fruit, seed and peel did not show any antimicrobial activity when examined against a wide range of bacteria and fungi including *Staphylococcus aureus*, *E. coli*, *Bacillus subtilis* and *Candida albicans* (Moniharapon and Hashinaga, 2004). The methanol extract of *Kigelia africana* (belonging to the family Bignoniaceae) presented a higher activity than the aqueous and chloroform extracts with moderate activity against *Staph aureus* and *E. coli*.

The antimicrobial activity may involve complex mechanisms like the inhibition of the synthesis of cell

wall, cell membrane, nucleic acids and protein as well as the inhibition of the metabolism of nucleic acids (Oyaizu *et al*., 2003).

The antibacterial activity of plant extracts can be attributed not only to a single bioactive principle but also in concert action with other compounds (Sunayana *et al*., 2003). A number of phytochemicals have been studied for their antimicrobial activity and found potentially useful against infectious diseases. The chemical structure of the antimicrobial agents found in higher plants belong to most commonly encountered classes of higher plants secondary metabolites such as flavonoids (Watchter *et al*., 1999), terpens (Conveney *et al*., 1985), terpenoids (Osawa *et al*., 1990; Habibi *et al*., 2000) and phenolic acids (Fernandez *et al*., 1996).

CONCLUSION

From this study it was concluded that the spectrum of antimicrobial activity of the investigated methanolic and aqueous extracts of *Cola acuminata* seed against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonae* in addition to the yeast *Candida albicans*, was found comparable to the reference antibacterial (Gentamicin) and antifungal (Nystatin) drugs. The petroleum ether extract of the same plant was found to have no inhibitory activity against both tested bacteria and yeast.

This investigation may justify the use of this plant in the local traditional medicine. Detailed *in vitro* studies utilized the characterized active principle of the plant are necessary for assessing these antimicrobial constituents.

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