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Proximate and Antimicrobial Properties of *Garcinia kola*; a Significant Evidence of a Functional Food Snack

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Abstract: ***Garcinia kola an economic plant is widely valued in West and central Africa for its edible nuts as well as for ethno-therapeutics applications. Macronutrients and antimicrobial potential of the seed were investigated. Proximate composition was done and the active constituent extracted by water and acetone extractions at different concentrations. Bacteria isolate were tested to determine the potency of the extract as an antimicrobial agent. The extract of Garcinia kola was concentrated and varying dosage was used to confirm the susceptibility profile of six bacterial isolates. The result showed that the aqueous extracts (hot and cold) exhibited higher antimicrobial activity at 200mg/ml, with zone of inhibition between 18-24 mm for hot water and 5-19.4 mm for cold water extracts respectively. Graded acetone extract was found to exhibit more significant inhibition against the isolates. The highest zone of inhibition was observed at 200.00 mg/ml in all composition of acetone, highest inhibition of 28.00 mm was seen on absolute acetone for Staphylococcus aureus; 22.00 mm at 80 % in both Staphylococcus aureus and Klebsiella pneumonae; at 60 % inhibition zone of 19 mm in Staphylococcus aureus and Klebsiella pneumonae. While the lowest zone 10.00 mm was seen at 06.25 mg/ml; 10.00mm at 12.50 and 06.25 mg/ml 80 % and10 mm at 25.00 mg/ml for 60 %. This work showed that G. kola has food value and antimicrobial properties, both aqueous and acetone extract has varying degree of antimicrobial properties relative to its concentration.***

Keywords: *Garcinia kola*, inhibition, antibiotics, susceptibility, extract

1. Introduction

Food bioactive compounds have been significant sources of nutraceuticals and an obvious evidence of functional food. The Scientific research has shown how active and effective are the phytochemicals as therapeutic agent (Bodeker, 2000; Espin *et al.,* 2007). Functional foods, nutraceuticals are food natural bioactive, chemical compounds that have health promoting, disease preventing or medicinal properties. There is a direct relationship between foods and health, significant evidence of which has been shown in various scientific studies (Dureja *et al.,* 2003; Vicentini *at al.,* 2016). Increase in demand for such foods can be explained by increasing cost of healthcare, steady increase in life expectancy and the desire to improve quality life (Kaur and Das, 2011; Vicentini *et al.,* 2016). A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially on one or more target functions in the body, beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and reduction of risk of disease (Reis *et al.,* 2017)

There is increase awareness of phytochemicals health benefit, the impact of these plant derived substances on health is noted and have been used in treatment of different ailments thus has been documented (Espin *et al.,* 2007; Adesuyi *et al.,* 2012). Consumers' interest in health and well-being has redirect the attention of consumers to traditional snacks of plant origin and much more to herbs, nuts, spices and roots that are eaten sparingly and majorly at occasions. One of such nuts is *Garcinia kola*, this product is known to offers specific beneficial effect on health. The list of such product includes food ingredients such as nutmeg, alligator pepper, ginger and clove; these products may contain essential and non-essential nutrients. Some are leafy vegetable, nuts others are roots (Savithramma *et al.,* 2011; Salehi *et al.,* 2019).

Phytochemicals are non-nutritive plant chemicals that are widely present in plant food products, it has been confirmed to be bioactive, thereby contributing to human health (Lu, and Zhao, 2017). It is non-nutritive plant chemicals that have protective or disease preventive properties, this chemical substance have been shown to protect humans against disease as well as risk reduction for a variety of chronic or inflammatory conditions. Some of the well known phytochemicals include; lycopenes found in tomatoes, isoflavones in soy beans and flavonoids in fruits. (Adesuyi *et al.,* 2012). Group of polyphenolic compounds are abundantly present in human diet through plant food product especially those rich in spice, fruits and nuts. There are different types of flavonoids and each appears to have significant health value which include anti-inflammatory, ant-oxidant, antiviral, and anti-carcinogenic properties (McCann *et al.,* 2003; Somani *et al.,* 2015).

Epidemiological studies on the relationship between plant-derived food and disease risk have shown that food has a

direct impact on health (Alidadi *et al.,* 2020). It is generally accepted that plant derived foods such as wine, fruits, nuts, vegetables, grains, legumes, spices, etc. exert some beneficial effects on human health, particularly on age-related diseases. These benefits have been related to some phytochemicals constituents of plant based food of fruits, nuts, roots and vegetable origin. Among these plant derived chemicals are anthocyanins, proanthocyanides, flavanones, isoflavonones, resveratrol and ellagic acid. There are numbers of non-nutrient components of plant foods which are beneficial on the bases of their capacity to reduce the risk of chronic disease, these components of plant foods are the phytochemicals which has been confirm through different studies to exert a wide range of biological activities. Many of the plant-derived food include bitter cola (*Garcinia kola*), lemmon grass, ginger, nutmeg, and bitter leave (Gedikoglu *et al.,* 2019).

Garcinia kola (*G. kola*) is a widely cultivated tree that is highly valued in West and central Africa for its edible nuts. The extracts of the seed commonly known as bitter kola have been employed in Africa herbal medicine for treatment of illnesses such as laryngitis, liver diseases, cough, and diabetes. Bitter kola is edible seed, a stimulant, which belongs to a unique group of plants that help human being to withstand or adapt to stress by influencing multiple regulatory systems responsible for stimulus-response coupling such as the immune system and act also as a general anti-infective agent (Granado-Lorencio, and Hernández-Alvarez, 2016). *Garcinia kola* is an angiosperae, it belongs to the family guttiferae it is commonly called bitter cola. On chewing, bitter cola has an astringent and resinous taste somehow resemble the taste of coffee. Bitter kola is important in Africa ethno medicine, highly prized for its numerous medicinal use as well as it place in social functions. The use of bitter kola in traditional medicine is been documented since time immemorial, in resent time its application has been pronounced with application of modern methods to extract it active ingredient as concentrate (Atawodi *et al.,* 1995; Mythilypriya *et al.,* 2007; Nkono *et al.,* 2022).

The role played by *G. kola* in traditional ceremonies is central to its uses, because it is very important in all traditional engagements; in childbirth, marriage, chieftaincy ceremonies respectively (Adebayo and Oladele, 2012; Durand *et al.,* 2015; Yogom *et al.,* 2020). Though *G. kola* is valued for it medicinal application, it is eaten raw, also for it nutritional value, it is often presented in ceremonies in its raw form as snack, therefore, as the current research target its antimicrobial properties, it is also necessary to investigate it food value and its nutritional compositions (Maňourová *et al.,* 2019). In line with sustainable development goal of the united nation, good health is achievable when our foods, *Garcinia kola* inclusive serve as therapeutic agent to fight off many of the agents of ill health, the goal will be better achieved.

2. Materials and Methods

2.1 Materials

Fresh seed of *G. kola* were purchased from Oja-oba market in Iloring, Kwara State. Healthy seeds without sigh of damage were selected, peeled; sliced and dried over a period of 5-6 days at 40 ^{o}C in an oven. When the sample was dried to 15 % it was milled into fine powder with the aid of Kenwood blender BL 440.

2.2 Proximate Analysis

Proximate analysis was carried out following the method described by AOAC (2005), the proximate value investigated are: Ash content, fat content, crude fiber, crude protein, moisture content.

2.3 Microbial isolation

Microbial isolate which were *Staphylococcus aureus, Escherichia coli, Streptococcus pneumonia, Haemophilus influenza, klebsilla pneumonia, Bacillus subtilis* were obtained from previous research on hospital waste management study in ogbomoso, metropolis, Oyo State, Nigeria (Adeoye *et al.,* 2018).

2.4 Preparation of the crude seed extract

The *Garcinia kola* seed already sorted and dried was made in to powder by blending, the powder was prepared kept in cellophane bags and stored to be used for the analysis.

2.5 Aqueous extraction of active ingredients in *G. kola* (cold water)

Extraction was done with 100 g of powder soaked in 300 ml of sterile distilled water accompany with thorough agitation for after 48 h of soaking. The liquid extract was separate from the chaff with the aid of Whatman filter paper No 1. It was concentrated by freeze drying at-50 ^{o}C under vacuum. The concentrated extract was later store in air tight bottle at-4 ^{o}C.

2.5.1 Aqueous extraction of active ingredients in *G. kola* (hot water)

Hot water extraction of active ingredient of 100 g of prepared powder was done; using 30 minutes soaked *Garcinia kola* powder. It was similarly agitated for 48 h before extraction. The extract was done with similar procedure as for the cold extract. The filtrated sample likewise was obtained and stored under-4 ^{o}C in airtight clean bottle.

2.5.2 Extraction of active ingredients using Acetone (Organic solvent)

Exactly 100 g of sample was soaked in 300 ml of graded concentration of acetone at varying percentage: 100, 80, 60, 40, and 20 % respectively. Each of the samples was mixed and agitated similarly and allowed to stay for 48 h. the samples were then filtered, the filtrate was concentrated using rotary evaporator at 50 ^{o}C, the resulted concentrate was then stored similarly at-4 ^{o}C.

2.6 Preparation of graded concentration of the samples

From each extracted samples, accurately 6.25, 12.5, 25, 50, 100 and 200 mg respectively weighed and make into solution of 1ml of their respective solvents of extraction for proper dissolution and applications.

2.7 Culture media preparation

The media used for the culture of the isolates was Nutrient Agar, Mueller Hinton Agar and Nutrient broth. The media were prepare according to the manufacturer' specification and were sterilized in an autoclave for 15 min at 121^{o}C.

2.8 Antibacterial test of the plant extract with pour plate technique

Culture of each of the organism was prepared by taken a loop full of the organism from the active stock and inoculated each into the already prepared sterile 5 ml nutrient broth, each incubated for 18 – 24 h at 37 ^{o}C. From the incubated broth serial dilution was done to obtain 10^{-2} dilution ratio of the organism. Nutrient agar was prepared and from the broth medium of each diluted broths, 0.2 ml was taken into the respective petri-dishes, agar were poured and allow to solidify for about 45 min., Using a sterile cork borer of 8 mm diameter, wells were made on the solidified nutrient agar, according to the number of graded concentration of the plant extracted samples. In each well, the different graded concentration of the plant extract sample were poured; repeatedly in duplicates. The plates were allowed to stay on the bench for 2 h to allow diffusion. The plates were then incubated uprightly in the incubator for 24 h at 37 ^{o}C.

3. Results and Discussion

Proximate evaluation of *Gacinia kola* carried out was shown in Table 2, the result obtained gave evidence that the seed is rich in carbohydrate; 88.35 %, also *Gasinia kola* is a good source of fiber, it also contains protein, crude fat. The moisture content is relative to season and time of analyzis, fresh nut will have high moisture content (Adesuyi et al., 2012; Adeniyi *et al.,* 2015; Dah-Nouvlessounon *et al.,* 2015). This result is comparable to result obtained my previous author; Adesuyi *et al.* (2012) obtained similar quantity of macronutrients in a sample of *G. kola*,

Table 1: Proximate analysis of *Gasinia kola*

	Nutrients	Percentage
i	Carbohydrate	88.35
ii	Protein	1.86
iii	Crude fibre	1.23
iv	Ash	0.47
v	Crude fat	0.19
vi	Moisture	7.8
	Total	100

The percentage crude extract yield of *Garcinial kola* seed was shown in Table 1.0. The table shows that the percentage yield obtained was maximum in absolute acetone with 18.61 % extraction; the minimum was obtained from the lowest dilution of 20 % which was 15.19 %. While hot water extraction gave 12.76 % that of cold water was the least value obtained. The ability of acetone as solvent is evidently shown here, with concentration as a factor that influence recovery (Li *et al*., 2006; Sepahpour *et al.,* 2018) It has been demonstrated in terms of high efficiency that extraction using organic solvent has high throughput and solvent extraction has been employed in industrial extraction in several ways (Afolayan *et al.,* 2020; Tao *et al.,* 2021; Mukhopadhyay *et al.,* 2022).

Table 2: Garcinia kola seed extract (%) using acetone and water as solvents

S/N	Extraction solution	Extract yield	Percentage yield (%)
1	100 % Acetone	18.61	18.61.
2	80 %	17.03	17.03
3	60 %	16.92	16.92
4	40 %	16.27	16.27
5	20 %	15.19	15.19
6	Hot water	12.72	12.76
7	Cold water	12.02	12.02

The result of the antimicrobial strength of Gacinial kola was presented in Table 3. Seven organisms were exposed to the extract obtained from the Gasinia kola sample, the organisms were: *Staphylococcus aureus, Escherichial. coli, B. subtilis, Klebsiella pneumonia, Haemophilus influenza and Streptococcus pneumonia*. With proper examination of the reaction and growth of the organisms when exposed to the extract in the well on the media, the antimicrobial activity of the aqueous extract against the tested microorganisms showed zone of inhibition. The potency of antibiotics to inhibit the growth of the organisms was indicated by the appearance of clear zone in circumference round the well otherwise its resistance. Similar investigation has been carried out by researchers using zone of inhibition to estimate susceptibility of the isolates (Adeoye *et al.,* 2018; Adeoye *et al*., 2020; Ojo *et al.,* 2021) it was deduced that plant extract when properly obtained is a veritable tools for fighting microbial infection (Tekwu *et al.,* 2012; Jahani *et al.,* 2016). However, their sensitivity is shown to be relative to concentration of the extract, the higher the concentrations the wider the inhibition zones (Bhargav *et al*., 2016). Similar report had been reported by several Authors on the antimicrobial activities of plant extracts (Gonelimali *et al.,* 2018; Nieto and Castillo, 2018; Daoud *et al.,* 2019). Zone of inhibition as seen in cold water extraction was 10.0 mm, at 6.25 mg/ml *E. coli* while at higher concentration of 200mg/ml was 18.0 mm; hot water extract produced 17.0 mm at 6.25 mg/ml while at 200 mg/ml produced 21.5 mm. The lowest zone inhibition 10 mm was observed in *E. coli* at the 6.25 mg the lowest concentration. The widest zone was observed in *Bacilus subtilis and Staph aureus* 24.0 mm at 200 mg/ml. The finding here is corroborating some previous research on antimicrobial properties of *G. kola* (Arekemase *et al.,* 2012; Sabo *et al.,* 2020).

Table 3: Sensitivity pattern of microorganism isolates (Zone of inhibition) in cold and hot water extract of *Gasinia kola (mm)*

	Cold water extraction						Hot water extraction					
Concentration (mg/ml)	6.25	12.5	25.00	50.0	100.0	200	6.25	12.50	25.00	50.0	100	200
Microorgnism	Zone of inhibition in mm											
Stap. aureus	11.0	11.0	13.0	15.0	16.0	19.4	18.4	20.0	22.0	23.0	23.5	24.0
E. coli	10.0	10.5	12.0	14.5	16.0	18.0	17.0	18.0	20.0	21.0	21.0	21.5
Klebsiella pneumoniae	-	-	-	-	-	5.0	12.0	15.0	16.0	19.0	20.0	20.5
Strep. pneumoniae	-	-	-	12.0	12.0	16.0	16.0	18.0	21.0	21.5	21.5	22.0
Haemophilus influenza	-	-	-	-	-	-	12.0	16.0	17.0	17.0	18.0	18.0
Bacillus subtilis	10.6	11.0	12.0	13.0	14.0	16.0	11.3	14.0	16.0	18.0	21.0	24.0

-; no inhibition; *Staph, Staphylococcus, E. coli, Escherichial coli; Srep, Streptococcus*

The organisms' pattern of growth in absolute acetone extract was presented in Table 4. The concentration of the extract was in mg/ml with the highest and lowest concentration of 200 mg/ml and 6.26 mg/ml respectively, the widest zone of 28.0 gm/ml observed in *Staph. aureus* and the least zone of 24.0 mm were obtained in *E. coli, Strep. Pneumoniae, Haemophilus influencer, Bacillus subtiis* respectively. At concentration of 100.0 gm/ml the highest zone of inhibition was 24 mm and the least was 18 mm. at 50 mg/ml; the highest and the lowest zone of inhibition were 20.0 and 16.0 mm respectively. At 25 gm/ml the highest and the lowest were 18.0 mm and 14.0 mm respectively. At 12.50 gm/ml; the highest and lowest zone of inhibition were 16.0 mm and 10.0 mm respectively the last of the tested concentration was 6.25 gm/ ml, the highest and lowest zone of inhibition were 12.0 mm and 10.0 mm, there is no inhibition at that concentration on *Strep. pneumoniae*.

Table 4: Sensitivity pattern of microorganism isolates in absolute acetone extract of Gasinia kola (Zone of inhibition; mm)

	Concentration mg/ml					
Microoganism	200.00	100.00	50.00	25.00	12.50	6.25
Staph. aureus	28. .00	24.00	20.00	18.0	16.0	12.0
E. coli	24.00	20.00	18.00	16.0	14.0	12.0
Klebsiella pneumoniae	25.00	22.00	19.00	17.0	15.0	11.0
Strep. pneumoniae	24.00	18.00	16.00	14.0	10.0	-
Haemophilus influenza	24.00	20.00	18.00	14.0	12.0	10.0
Bacillus subtilis	24.00	20.00	18.00	14.0	12.0	10.0

-; no inhibition observed

At lower concentration of acetone (80 %) as shown in Table 5, the process was repeated on the isolated organisms the zone of inhibition is seen to be reduced. The highest was 22 mm as obtained in *Staph aureus* at 200 mg/ml, the least was 10 mg/ml this was observed in lower concentration of 25.00, 12.50, 6.25 mg/ml respectively, these shows that the decline concentrations of the extract lower its sensitivity. At the lowest concentration 6.25 mg/ml there are no inhibitions for *E. coli, Klebsiella, Strep. pneumonia, Haemophilus influenza.*

Table 5: Sensitivity pattern of microorganism isolates in 80 % acetone extract of Gasinia kola (Zone of inhibition; mm)

Concentration mg/ml						
Microoganism	200.00	100.00	50.0.0	25.00	12.50	6.25
Staph. aureus	22.0	19.0	17.0	14.0	12.0	10.0
E. coli	20.0	18.0	14.0	12.0	10.0	-
Klebsiella pneumonea	22.0	18.0	17.0	14.0	11.0	-
Strep. pneumoniae	16.0	14.0	12.0	10.0	-	-
Haemophilus influenza	20.0	18.0	14.0	12.0	10.0	-
Bacillus subtilis	20.0	18.0	16.0	14.0	12.0	10.0

To further confirm the effect of concentration on the sensitivity of the extract, the lower concentration 60 % acetone showed more resistance and lower sensitivity, as presented in Table 6. The highest inhibition was seen in *Staph. aureus,* and *Klebsielle pneumonia,* 19.0 mm respectively at 200 mg/ml, the lowest susceptibility was 6.0 mm as shown in concentration of 25 mg/ml. There was more resistance as the concentration of the extract was lowered, which reflected in no zone of inhibition for *Staph. aureus* and rest of the isolates at 6.25 mg/ml while at 12.50 mg/ml only *Staph aureus* was inhibited at the least level. The solvent; acetone as control experiment, was observed to have no inhibitory effect on the growth of the isolate on the other hand 10 µg/ml Gentamicin had very notable inhibitory effect as shown in Table 7.

Table 6: Sensitivity pattern of microorganism isolates in 60 % acetone extract of Gasinia kola (Zone of inhibition; mm)

Concentration mg/ml						
Microoganism	200	100	50.0.0	25	12.5	6.25
Staph. aureus	19	17	14	12	10	-
E. coli	18	14	12	10	-	-
Klebsiella pneumonae.	19	17	13	6	-	-
Strep. pneumoniae	16	14	12	10	-	-
Haemophilus influenza	18	14	12	10	-	-
Bacillus subtilis	18	14	12	10	-	-

Table 7: Sensitivity pattern of microorganism isolates in 10 µg/ml Gentamicin and acetone as control (Zone of inhibition; mm)

Microorganism	Inhibition zone (10 µg/ml Gentamicin)	Acetone
Staph. aureus	39	-
E. coli	38	-
Klebsiella pneumoniae	39	-
Strep. pneumoniae	40	-
Haemophilus influenza	40	-
Bacillus subtilis	38	-

Key: Gentamicin + control; Acetone: control, –: no inhibition

4. Conclusion

It can be deduced from the result obtained from the proximate analysis of the *G. kola* that it is a good source of macronutrients, with appreciable carbohydrate value. Consumption of it will actually meet some nutritional needs and enhance good health, thus given assurance of meeting goal number 3 of SDG. The ability of the extract to inhibit the growth of microorganism is evident in the result obtained. The sensitivity of the extract will be enhanced when appropriate concentration is applied. All the isolate tested in the work were pathogenic organism which has been implicated in the clinical test, their susceptibility to local snack that has been employed for ethno-medicine, G. kola as a snack is a functional food because it has been demonstrated in several fora, and it is been proof here to have beneficial health value beyond just a snack for occasion. From this research work, *G. kola* extract exhibited antimicrobial activity ranging from 6.25 – 200 mg/ml, the higher concentration of the extract exerted higher potency.

Authors Contributions:

Alabi WO: formal analysis. Olanipekun BF: Conceptualization, Editing. Adeoye AO: Investigation, Methodology, Validation. Alabi WO, Adeoye AO: Investigation, Validation, Adeoye AO: Writing of the Manuscript and Investigation. Adeoye AO: Manuscript writing, Editing. Ajani RA: Validation, Investigation. Ohijeagbon OR: Validation, Investigation.

Competing Interests-no competing interest

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Ethical Issues and consent

The work involves the use of hospital waste of human origin, collection procedure were performed in strict compliance with relevant laws and institutions guideline, approval of relevant authority concerned were obtained

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