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Research Article

Comparative Study of Antibacterial Activities of Ethanol Extracts of the Bark and Seeds of *Garcinia kola* and *Caricapapaya*

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ABSTRACT: A comparative study of the antimicrobial activities of the bark and seed extracts of *Garcinia kola* and *Carica papaya* were tested using the Agar well diffusion method on eight bacterial strains - *Staphylococcus aureus; Salmonella typhi B; Shigelladysenteria; Pseudomonas aeruginosa; Serratiamarcescens; Pseudomonas fluorescens; Proteus vulgaris;* and *Bacillus subtillis.* Phytochemical screening shows that both bark and seed of the two plants contain reducing sugars, phenols and alkaloids whereas the pawpaw extracts contained tannins which were absent in the bitter kola plant part. *Garcinakola* on the other hand has saponin that was not present in *Caricapapaya.* The *Garcinia* seed ethanol extract manifested the best inhibitory activities against the test bacteria, producing inhibition zones ranging from 12–23mm. There was no resistance from any of the test bacteria. The pawpaw seed extract was also effective against the test bacteria. The inhibition zone observed ranged from 11-16mm. The ethanol extract of both plants were active against *Staphylococcus aureus, Shigelladysenteria, Pseudomonas fluorescens*; and *Salmonella typhi* B. Pawpaw leaf ethanolic extract significantly inhibited *Pseudomiasaeruginosa,* while Garcinia seeds ethanolic extract also inhibited *Bacillus substilis.* The activity index of ethanol extract of *Garcinia kola* seed was higher than that of pawpaw seed when both gentamicin and tetracycline were used as a standard antibiotics. The aqueous extract of both plant parts were not as effective as the ethanol extract. The activities of these medicinal plants against the tested bacterial species in this study justified their common use in African traditional medicine in the treatment of various ailments.

Keyword: *Garcinia kola*, *Carica papaya*, Aqueous extract, ethanol extract, bacteria

INTRODUCTION

The search for healing powers in plants is as old as man. People in all continents of the world have long applied poultices and imbibed infusions of hundreds (if not thousands) of indigenous plants dating back to pre-historical period (Duke and Wain, 1981; Nostro *et al*., 2000). Till date, natural plants of various types are used in traditional African medicine for providing healing to various ailments even before and after the spread of modern and scientific medicine. Attempts have been made to justify, on a scientific basis, the practice of African traditional medicine. Hence some active components of medicinal plants often used which produces certain anti-microbial properties have been identified. *Garciniakola* is commonly known as 'Bitter kola' is used extensively in various parts of Africa for the treatment of various diseases. Seeds of the plant are used in Southern Nigeria and parts of West Africa as masticatory despite its bitter taste (Iwu *et al*., 1987). It is also used for cultural and social ceremonial purpose, traditional hospitalities and worship of ancestral gods among traditional worshipers in some part of West Africa (Iwu, 1993). Bitter kola is one of those few herbal plant referred to as 'wonder plant' because of its' extensive use in the treatment of various disease in Africa traditional medicine. It is eaten raw as adjuvant of true kola and quoted as Guinea worm remedy and as a vermifuge (Sofowora, 1982). It prevents colics and particularly known to improve

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singing voice, due to its effectiveness for bronchitis and throat troubles (Iwu, 1993). The uses of other parts of *Garcinia kola* tree have been documented (Adaramoye *et al.*, 2005; Sofowora, 1982).

Carica papaya Linn: is commonly called pawpaw, and it is native to tropical countries. The vegetative parts of papaya plant have enormous medicinal uses in various parts of Africa. The seeds of pawpaw have been reported to cure cough when eaten raw in some parts of Nigeria (Gills, 1992). Studies have shown that air-dried seeds of pawpaw when administered orally decreased Dirofilariaimmitis in dogs (Ghosh, *et al*., 1998). An infusion of the fruits in water when unripe is used as an effective remedy for the treatment of jaundice resulting from yellow fever infection in babies in Nigeria. The fruit when ripe has an attractive colour due to the presence of carotenoids which is a possible anticancer compound and protectors of the gastric mucosa. It is also a source of vitamins A and C.

Due to the immense application of various parts of these two plants in treatment of different ailments in Africa traditional medicine, this study therefore aimed at comparing the effectiveness of different parts of the plants against some opportunistic and common pathogenic bacteria, and also investigate the physiochemical component of these medicinal plants.

MATERIALS AND METHODS

Garcinia kola bark and seeds were obtained from a local forest in Sagamu while pawpaw seeds were obtained from fruits bought from Bodija market in Ibadan Oyo-State. The leaves of pawpaw were obtained from the trees in the surroundings of the Department of Botany and Microbiology, University of Ibadan. The plants parts were authenticated at the herbarium of the Department of Botany and Microbiology, University of Ibadan. The various plant parts were sun dried for several days and later oven-dried at 70^{o}C for 24 hours and then blended into powder. They are thereafter packed separately into clean polythene bags and labeled accordingly.

Extraction Method

The extraction of the plant parts was carried out using ethanol and distilled water as extracting solvents. The cold maceration extraction method of Cowan (1999) was used. Fifty grams of each part of the plants was weighed and dissolved in 1000ml of the extracting solvent inside a 2 litre conical flask and covered with parafilm (Ogunjobi and Nndozie, 2004). The flasks were shaken vigorously at 30 minutes interval and left to stand for 24 hours at room temperature. The resultant mixture was then filtered with whatman's No. 4 filter paper and cotton wool to remove particles of plant sample. The clear solution obtained was distilled at 65^{o}C under low pressure on a steam bath. The semi solid concentrations of the extracts were then collected in sterile pre-weighed screw capped bottles and labeled accordingly (Ogunjobi *et al*., 2007). The extracts were store at 4^{o}C until when needed.

Anti-Microbial Sensitivity Test

The agar well method of the agar diffusion technique was used in this study to determine the antibacterial activity of the plant extracts as described by Adegoke and Adebayo-Tayo (2009). The Nutrient agar (Diffco) used was prepared according to manufacturer specification and the extract were tested against eight different bacteria species listed in Table 1.

A cork borer of 5 mm was used to make a hole on the agar plate. The extract was added to fill the hole bored by 5 mm cork borer in the inoculated agar. Both plant extract were not diluted to find the appropriate dilution for it effectiveness because local herbal practitioners do not dilute them before use.

Table 1. Test microorganisms used and their sources

Organisms	Code	Source
Staphylococcus aureus	ATCC 2593	Department of Bot. & Micro. U.I.
Salmonella typhi B	AP 22096	Laboratory Stock
Shigella dysenteria	AP 22433	Laboratory Stock
Psedomonasaeruginosa	NCIB 950	Department of Micro. OAU, Ile-Ife
Serratiamarcescens	NCIB 1377	Department of Micro. OAU, Ile-Ife
Pseudomonas fluorescens	NCIB 3756	Department of Micro. OAU, Ile-Ife
Proteus vulgaris	NCIB 67	Department of Micro. OAU, Ile-Ife
Bacillus subtilis	Laboratory culture	Laboratory Stock

The plates were made in duplicate; gentamicin and tetracycline were employed as a standard drug to compare the effectiveness of the plant extracts.All plates were incubated at 37^{o}C for 24 h. The diameter of the zones of inhibition in the duplicate plates was measured by calculating the difference between core borer (5 mm) and the diameters of inhibition as described by Hewitt and Vincent (1989).The activity indices were calculated as the division of zone of inhibition of the extract by that of the standard drugs following the method of Singh *et al*. (2002). The results were presented as the means values of the duplicate plates

Phytochemical Screening

This was carried out by using a modified method of Lajubutu *et al*. (1995) and the following were tested for, Alkaloids, Tannins, Saponin, Anthraquinone, Glycoside, & phenols

To test for alkaloids, about 0.5 g of the extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. A few drops of Dragendorff's reagent were used to treat 1 ml of the filterate. Turbidity or precipitation with this reagent was taken as evidence for the presence of alkaloids. Exactly 0.5 g of the extract was dissolved in distilled water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for saponins. Also, the presence of tannins was determined by dissolving 0.5 g of the extract in distilled water and about 10 ml of bromine water added. Decolourization of bromine water indicated the presence of tannins. Borntrager's test was used for detecting the presence of anthraquinones. In this case 0.5 g of the plant extract was shaken with benzene layer separated and half of its own volume of 10% ammonia solution added. A pink, red or violet coloration in the ammoniacal phase indicated the presence of anthraquinone. The presence of cardiac glycosides and phenols was established by Salkowski test, Liberman's test, and Keller-Killani test (Sofowora , 1993; Trease and Evans, 2002).

RESULTS

The result of the phytochemical screening shown in Table 2 revealed that the two plant parts contained reducing sugars, phenol and alkaloids whereas the pawpaw plant parts contained tannins which was absent in the bitter cola plant part. *Garcinakola* on the other hand has saponin that was not present in *Caricapapaya*. The result of the anti-microbial sensitivity test against the eight different bacteria species shown in Table 3 depicts that the *Garcinia* seed ethanol extract was best, producing inhibition zones ranging from 12–23mm. There was no resistance from any of the test bacteria. The pawpaw seed extract was also effective against the test bacteria. The inhibition zone observed ranged from 11-16mm. The activity of the *Garcinia* back ethanol extract against the test bacteria was also comparatively effective, but was not active against *Pseudomonas aeruginosa*. Nevertheless, a zone of inhibition ranged between 15-20mm was observed. The least activity against the test bacteria was that of *Garcinia* back aqueous extract. The test bacteria were quite resistant against the extract.

However the extract was effective against *S. aureus* and *S. marcescens*, producing 11mm and 14mm zone of inhibition respectively.

Table 2:

Phytochemical components of bark and seed extracts of *Garcinia kola* and *Carica papaya*

Test Compounds						
Sample	Reducing Sugars	Saponin	**Tannins**	Anthraguinones	Phenol	Alkaloids
PLE	-	-	+	-	+	+
PLW	-	-	+	-	+	+
PSE	-	-	+	-	+	+
PSW	+	-	+	-	-	+
GSE	+	+	-	-	+	-
GSW	+	+	-	-	+	+
GBE	+	+	-	-	+	-
GBW	+	+	-	-	+	+

PLW- Pawpaw leaf aqueous extract; PLE- Pawpaw leaf ethanolic extract
PSW- Pawpaw seed aqueous extract; PSE- Pawpaw seed ethanolic extract
GSE- Garcinia seed ethanolic extract; GSW- Garcinia seed aqueous extract
GBE- Garcinia back ethanolic extract; GBW- Garcinia back aqueous extract.

Table 3:

The diameter of zone of inhibition of each extracts on the test organisms

Zone of Inhibiton (mm)												
Organisms	PLW	PLE	PSW	PSE	GBE	GBW	GSW	GSE	H_2O	Ethanol	Gentamicin	Tetracyline
S. aureus	5.0±0.5	11.0±0.4	6.0±0.5	12±0.4	18.0±0.2	11.0±0.1	13.0±0.8	23.0±0.9	-	5.0±0.1	26±0.2	21±0.4
S. dysenteria	2.0±0.8	13.0±0.6	8.0±0.2	14±1.0	16.0±1.2	-	-	19.0±0.7	-	8.0±0.6	19±0.1	16±0.5
P. aeruginosa	-	24.0±0.2	12.0±0.4	15.0±1.2	-	-	8.0±0.4	12.5±0.3	-	7.5±0.4	16±0.3	9.0±0.6
P. fluorescens	4.0±1.0	16.0±0.5	-	13.0±1.0	20.0±0.4	-	9.0±0.3	19.4±0.5	-	5.6±0.2	22±0.1	18±0.2
S. marcescens	-	-	-	12±0.4	16.0±0.5	14±0.3	-	16.8±1.1	-	9.0±0.5	21±0.4	21±0.3
P. vulgaris	-	-	6.0±0.8	13.0±0.2	19.0±0.3	-	11.0±0.5	18.0±0.6	-	8.5±0.3	23±0.2	17±0.1
B. Subtilis	-	-	-	11.0±0.2	15.0±0.1	-	-	21.7±1.4	-	7.0±0.2	24±0.1	20±0.2
S. typhi	3.0±0.8	13.0±0.6	-	16.0±0.2	17.0±0.3	-	15±0.6	21.±1.3	-	1.0±0.1	23±0.3	16±0.3

- = No Inhibition

PLW - Pawpaw leaf aqueous extract *PLE - Pawpaw leaf ethanolic extract* *PSW - Pawpaw seed aqueous extract*
PSE - Pawpaw seed ethanolic extract *GSE - Garcinia seed ethanolic extract* *GSW - Garcinia seed aqueous extract*
GBE - Garcinia back ethanolic extract GBW - Garcinia back aqueous extract

Table 4:

The activity index of the extract of the both plants in comparison with standard antibiotic (Gentamicin)

Activity index									
Organisms	PLW	PLE	PSW	PSE	GBE	GBW	GSW	GSE	GENTAMICIN
S. aureus	0.19	0.42	0.23	0.46	0.86	0.42	0.50	0.88	26±0.2
S. dysenteria	0.11	0.68	0.42	073	0.84	-	-	1.00	19±0.1
P. aeruginosa		1.50	0.75	0.94	-	-	0.50	0.75	16±0.3
P. fluorescens	0.18	0.73	-	059	0.90	-	0.41	0.86	22±0.1
S. marcescens		-	-	0.57	0.76	0.67	-	0.76	21±0.4
P. vulgaris		-	0.26	0.57	0.83	-	0.48	0.78	23±0.2
B. Subtilis		-	-	0.46	0.63	-	-	0.88	24±0.1
S. typhi	0.13	0.57	-	0.70	0.74	-	0.65	0.91	23±0.3

Table 5:
The activity index of the extract of the both plants in comparison with standard antibiotic (Tetracyline)

Activity Index									
Organisms	PLW	PLE	PSW	PSE	GBE	GBW	GSW	GSE	TETRACYLINE
S. aureus	0.24	0.52	0.29	0.57	0.86	0.52	0.62	1.10	21±0.4
S. dysenteria	0.13	0.81	0.50	0.88	1.00	---	---	1.19	16±0.5
P. aeruginosa	-	2.67	1.33	1.67	---	---	0.89	1.39	9.0±0.6
P. fluorescens	0.22	0.89	-	0.72	1.11	---	0.50	1.07	18±0.2
S. marcescens	-	-	-	0.57	0.76	0.67	---	0.80	21±0.3
P. vulgaris	-	-	0.35	0.77	1.12	---	0.65	1.06	17±0.1
B. Subtilis	-	-	-	0.55	0.75	---	---	1.09	20±0.2
S. typhi	0.19	0.81	-	1.00	1.06	---	0.94	1.31	16±0.3

Table 4 shows the activity index of the plant extracts in comparison with gentamicin, a standard antibiotic. The activity index of Garcinia seed ethanolic extract seems to be quite high. A range of 0.75-1.00 was observed. Likewise, the activity index of pawpaw seed ethanolic extract, which ranges from 0.46-0.94. However, the highest activity index produced was 1.50 by papaw leaf ethanolic extract against *P. aeruginosa.* While the least produced was 0.11 by pawpaw leaf aqueous extract against *S. dysenteria***.**Table 5 shows the activity index of the plant extracts in comparison with Tetracyline. The activity index of garcinia seed ethanolic extract seems to be quite high. A range of 0.80-1.39 was observed. Likewise, the activity index of pawpaw seed ethanolic extract, which ranges from 0.55-1.67. However, the highest activity index produced was 1.67 by papaw seed ethanolic extract against *P. aeruginosa.* While the least produced was 0.13 by pawpaw leaf aqueous extract against *S. dysenteria.*

DISCUSSION

The results obtained from this study revealed that both plant parts contained bioactive agents that contain antimicrobial properties against both Gram positive and negative bacterial. This work showed that the leaves and seeds extract of *Caricapapaya* have inhibitory effect on *Staphylococcus aureus, Shigelladysenteria, Pseudomonas aeruginosa, Pseudomonas fluorescens and Salmonella typhi.* It is obviously interesting to observe the result of high antibacterial effects of the ethanol extract of pawpaw seeds on all the test bacteria.
In general, the ethanol extract of the plant parts obviously effective than the aqueous extracts. It is likely that the active constituents of the plant were better extracted with ethanol than with water, indicating that ethanol is a better solvent than water. The observation was similar to the report of Ogunjobi *et al*. (2004) and Ezeifeka *et al*. (2004) that reported the higher antimicrobial activities of ethanol extracts of plant parts that the aqueous extract.
Also, almost all the extract both plant parts showed appreciable level of potency against the commonest aetiology of enteric fever *Salmonella typhi*. Brooks *et al*. (2004) reported that enteric fever had mortality rate of 10 - 15% in developing countries. The extract of both plants may as well be put into use as therapy for treating the salmonella infection. Further pharmacological evaluations, toxicological studies and possible isolation of the active therapeutic ingredients will be of immense advantage in overcoming the menace of these bacterial diseases. The successful inhibition of these bacteria is a good development, especially when we consider the records of multi-resistance to various conventional antibiotics by bacteria over the years. The activity indices of the both plant parts with two conventional antibiotics showed that there was intrinsic composition of active ingredients in this plant that could be harnessed in combating the quandary of microbial infections in Africa. This finding justifies the traditional uses of these plant parts for therapeutic purposes. The plant has records of being use as analgesic, amebicide, antibacterial, cardiotonic, cholagogue, digestive, emenagogue, febrifuge, hypotensive, laxative, pectoral, stomachic, vermifuge and also effective against jaundice (Anibijuwon and Udeze, 2009). Anti cancer activity of better kola has been reported and the use in folklore remedies for the treatment of ailment such as liver disorders, hepatitis, diarrhea, laryngitis, bronchitis and gonorrhea are well known (Adaramoye *et al*., 2005; Ezeifeka *et al*., 2004; Okojie *et al*., 2009).
It could be however concluded that the demonstration of antimicrobial activity against both gram-negative and gram-positive bacteria is an indication that the plants are a potential source for production of drugs with a broad spectrum of activities. The results of the study also supports the traditional application of the plants and suggests that the plant extracts possess compounds with antibacterial properties that can be

used as antibacterial agents in novel drugs for the treatment of gastroenteritis, enteric fever, uretritis, and wound infections associated with the test bacteria.

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