

INVITRO ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *Carica papaya* AND *Azadirachta indica* LEAF AND STEM BARK EXTRACTS ON SELECTED CLINICAL ISOLATES.

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Abstract

The search for alternative sources of antibiotic is a global challenge due to the increase in the emergence of resistant strains. Plants have been in use in traditional medicine before the era of chemotherapeutics and about 80% of the global population still uses them. *Azadirachta indica* (neem) and *Carica papaya* are trees that have been found to possess antibacterial, antifungal, anti-inflammatory, anti-tumour properties and also used as a pesticide. In this work, antibacterial, phytochemical and antioxidant potentials of ethanolic and aqueous extracts of *Carica papaya* and *Azadirachta indica* leaf and stem bark was determined using antimicrobial sensitivity assay, minimum inhibitory concentration, minimum lethal concentration, Ferric reducing antioxidant power assay and Total antioxidant activity of extracts as indices. The test organisms were *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis* and *Staphylococcus aureus*. *Azadirachta indica* leaf water extract and *Azadirachta indica* stem bark ethanol extract showed a clear zone of inhibition ranging from $10\pm 0\text{mm}$ to $15.5\pm 0.71\text{mm}$ and $10\pm 0\text{mm}$ to $15.5\pm 2.12\text{mm}$ respectively against all four test isolates, while others extracts had clear zones of inhibition against at least three test isolates with inhibition zones ranging from $10.5\pm 0.71\text{mm}$ to $15\pm 1.41\text{mm}$. Ethanolic extract of *Carica papaya* leaf was active against *Bacillus subtilis* alone ($11.5\pm 0.71\text{mm}$). Some combined extracts expressed activity against all four isolate, while the highest individual extract inhibition zone was $15.5\pm 2.12\text{mm}$, combined extract was $18.5\pm 0.71\text{mm}$ against *Salmonella typhimurium*. All extracts had antioxidant activity and some of the phytochemicals present in the extracts include saponins, flavonoids, tannins, anthocyanin, betacyanin, quinones, cardiac glycoside, terpenoids, and phenols. However further research is still needed to identify the active phytochemicals and their concentrations in the extracts.

Keywords: *Azadirachta indica*, *Carica papaya*, Antimicrobial, phytochemical, antioxidants

Introduction

The use of plants in folk medicine predates microbiology and despite the increased scientific discoveries in the field of microbiology and modern medicine, use of plants as an alternative treatment is still very much prominent among low-income earners and rural dwellers. There has also been a recent increase in the use amidst the developed world too. In a report by the

World Health Organization, as high as 80% of global population subscribe mainly to traditional therapies that include the use of plant extracts or their active derivatives (WHO, 2012). The overuse or exposure to antimicrobials and poor hygienic conditions amongst others has resulted in selective effectiveness of antimicrobial agents and has been implicated in the rising emergence of drug and multi-drug

resistant pathogenic organisms which are currently a major public health concern across the globe. The last decade has witnessed a great drop in the discoveries of new chemotherapeutics able to combat emerging strains of pathogenic microbes. The pursuit of new chemotherapeutics for combating drug-resistant bacteria has led to the consideration of plants, mainly those with ethnopharmacological uses, have emerged as the major sources for recent discoveries of new chemotherapeutics. The broad biological diversity of plants are sources of a wide range of bioactive phytochemical molecules, acting through diverse mechanisms. This has resulted in the use of the extract from different parts of plants as sources of new chemotherapeutics or antibiotic agents, which have appeared to be of high importance since the emergence of resistant bacteria strains that have made treatment of infections with earlier established chemotherapeutics difficult. *Azadirachta indica* is among the array of medicinal plants that belong to the family Meliaceae and has been reported to be indigenous to South Asia but successfully cultivated across Africa including Nigeria. The medicinal efficiency of *A. indica* has been described by folk medicine practitioners over the years and some of which covers a wide range of medical conditions including treatment of stomach ulcers, fever, skin disorders, respiratory tract infections, rashes and boils, rheumatism, eye and ear infections, sore gums and throat, leprosy and diabetes.

Carica papaya is a member of the plant family *Caricaceae*, and various species belonging to this family have been used over the years for treatment of a variety of diseases. *Carica*

papaya is not just a tasty fruit but it is also a rich deposit of several bioactive compounds and antioxidant nutrients such as flavonoids; the B vitamins, vitamin C, carotenes and, folate and pantothenic acid; and the minerals, potassium, magnesium and fiber. The *papaya* is highly prized for its proteolytic enzymes which includes papain which is used in the treatment of sports injuries, other causes of trauma, and allergies.

Biochemically, the leaves of *papaya* are complex and produce numerous alkaloid and proteins with important pharmaceutical and industrial uses. Over the year's plant parts have been reported to be used as antimicrobial agents, in most cases, their extracts are used as infusions or oral administration. *Carica papaya* and *Azadirachta indica* leaf and bark have been used in the preparation of local herbs for treatment of several diseases. Bioactive compounds from plants are said to be a novel source of alternative therapeutic agents against infectious disease and a large number of phytochemicals agents have been extracted from plant parts like flowers, stem bark, stem, roots, fruits, seeds, leaves, fruit rind, and whole plants.

The aim of this research is to determine the antimicrobial, antioxidant and phytochemical properties of the aqueous and ethanolic crude extract of *Carica papaya* and *Azadirachta indica* leaves and stem bark on clinical isolates (*Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus subtilis* and *E. coli*) and thus advice on its usage.

Materials and Methods

Sample Collection and Processing

Leaf samples of *Carica papaya* and leaf and stem bark samples of *Azadirachta indica* were collected in the month of February

and March 2017 from Ota ($6^{\circ}40'45.7''N$ $3^{\circ}10'43.2''E$). The samples were transported to the Microbiology laboratory of Covenant University where they were identified by plant taxonomists in the Botany Unit of the Department of Biological Sciences, Covenant University, Ota. The samples were washed with potable water and air dried for 48-72 hours, shredded and oven dried at $40^{\circ}C$ for 24 hours to constant weight, blended to the powder using a mechanical grinder (VKP 1024B, Victorio, Utah, USA). The product was stored at $5^{\circ}C$ in an enclosed sterile glass container until used.

Collection and Storage of Microbial Isolates

The test organisms (*Staphylococcus aureus* subsp. *aureus* (ATCC[®] 25923TM), *Salmonella typhimurium* (ATCC[®] 4028), *Bacillus subtilis* subsp. *spizizenii* (ATCC[®] 6633TM) and *Escherichia coli* (ATCC[®] 25922TM) were obtained from Nigerian Institute of Medical Research (NIMR). Isolates were kept on nutrient agar slants and refrigerated at $4^{\circ}C$ until they were used. Prior to antimicrobial sensitivity assay, the isolates were subjected to basic cultural/morphological identification and biochemical tests for confirmation and purity test.

Preparation of the Extract

The extraction was carried out using water at $25\pm 2^{\circ}C$ and ethanol as solvents. Two hundred (200) g of the powdered leaf and stem bark were weighed and soaked in water and ethanol for 72 hours extraction with constant agitation at 100 rpm on a laboratory shaker (MaxQ400, Thermo Scientific, Massachusetts, USA). The samples were filtered and the filtrates were

concentrated in a rotary evaporator (RE300B, Stuart, Staffordshire, UK) at $40^{\circ}C$. The extracts were maintained at $5^{\circ}C$.

Antimicrobial Sensitivity Assay

Agar well diffusion technique as described by was used to determine the antimicrobial activity of the extracts. Müller-Hinton Agar (MHA) plates that have been checked for sterility were seeded with bacterial suspensions prepared from the fresh cultures with sterile distilled water with a turbidity of 0.5 McFarland Scale (1.5×10^8 cells/ml). Gentamicin sensitivity disc (10 μ g; Rapid Labs Ltd., UK) was used as positive control and the water and ethanol were negative controls. The extracts were tested on the isolates in uniform wells on the surface of the agar cut with a standard sterile cork borer of 9mm diameter. These tests were performed in triplicate and the plates were incubated at $37^{\circ}C$ for 18-24 hours and observed for zones of inhibition.

Determination of Minimum Inhibitory Concentration (MIC)

The standard agar dilution technique was used. Based on the results from the antimicrobial sensitivity assays, dilutions of each extract were made with concentrations ranging from 10 to 200mg/ml. The different concentration of the plant extract was introduced into sterile Petri dishes having one concentration per plate in replicates. Molten MHA was then poured into all the plate and swirled to mix with the extract at $45^{\circ}C$ before allowing setting. The proportion of media to extract was 18:2 respectively. Spot inoculation of the standardized bacterial cultures was made on the plates of Mueller-Hinton Agar (MHA) tintured with the varying concentration of plant extracts. The sterility of the MHA was tested setting

a plate without the test organism and plant extracts. The viability of the organisms was also tested using equivalent volumes of the media into which the extracts were added but the organisms. The MIC is the lowest concentration of the extract that prevents the growth of the particular isolate.

Determination of Minimum Lethal Concentration (MLC)

The MLCs was determined by selecting plates that show no growth during MIC determination. The lowest concentration that does not produce a single bacteria colony is considered as the MLC.

Phytochemical Screening

Phytochemicals examination of the extract was carried out for alkaloids, saponins, flavonoids and tannins, cardiac glycosides, glycoside, terpenoids, phenols, acids, triterpenoids quinones, and anthocyanin and betacyanin using the standard methods as described by Vanitha *et al.*, (2012).

The Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP was evaluated in the crude extracts of the plant samples using the method of Oyaizu (1986). A volume of 2.5 mL of the extract solution was mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture already prepared was then incubated at 50°C for 20 min. After that, 2.5 mL of 10% trichloroacetic acid (w/v) was added, then, the mixture was spun in a centrifuge at 116.272 g for 8 min (HERMEL Z 9 200A centrifuge). Aliquot 5ml of the supernatant was mixed with 5ml of deionized water and 1

ml of 0.1% of ferric chloride. Lastly, the absorbance readings of the solutions were documented as reading from the spectrophotometer (**Thermo Fisher Scientific, GeneSys, G105 UV-VIS, Madison, USA**) at 700 nm. The solution with high absorbance value expresses higher reducing power. In this procedure, EC₅₀ implies the concentration of extract in the solution which shows the absorbance of 0.5. It was extrapolated from the graph of absorbance at 700 nm against extract concentration in the solution. To compare extract reducing power, the standard was a methanolic solution of BHA.

The Total Antioxidant Activity

The total antioxidant activity of the extracts was performed according to Sharma *et al.*, (2016), 0.3ml of the extract was combined with 3ml reagent solution (0.6 M sulphuric acid, 28mm sodium phosphate and 4mM ammonium molybdate). The reaction mixture was capped and incubated at 95°C for 90 min. After cooling to room temperature; the absorbance was measured with the spectrophotometer (**Thermo Fisher Scientific, GeneSys, G105 UV-VIS, Madison, USA**) at 695nm against a blank (methanol 0.3ml). Ascorbic acid was taken as the standard.

Data analysis:

Statistical Package for Social Sciences (SPSS), version 20, was used for the data analysis. Results were expressed as the Mean \pm SD and tests of statistical significance were carried out using one-way analysis of variance (ANOVA). Statistical significance was defined as $P < 0.05$.

Results

Table 1 shows the antimicrobial activities of the crude extract and the control against the test organisms (*ATCC*[®] 25923*TM*), *Salmonella typhimurium* (*ATCC*[®] 4028), *Bacillus subtilis subsp. spizizenii* (*ATCC*[®] 6633*TM*) and *Escherichia coli* (*ATCC*[®] 25922*TM*). All extract but one were active against *Salmonella typhimurium* and performed better than the control antibiotic (Gentamicin). All extract had visible activity against *Bacillus subtilis*. Table 1 also reveals that the activity of combined crude extracts against the test organisms was either synergistic, additive or indifferent interaction and antagonistic in effect against the test bacteria. Table 2 shows results of minimum inhibition concentration assay for individual extract and combination of

extracts that showed zones of inhibition during the antimicrobial sensitivity assay. The lowest MIC values recorded were against *Bacillus subtilis* and *Salmonella typhimurium*. The phytochemical constituent of all plant extract was examined qualitatively and they are as listed in Table 3. The crude extract generated by water and ethanol for the same plant had a varying phytochemical composition in all plant samples worked on. The antioxidant activities of the plant extracts were carried out using FRAP and Total antioxidant capacity assay. And the absorbance of the assays is as represented in figures 1 and 2. The result shows that all extract has antioxidant potentials although some have better activity than others.

Table 1. Zones of inhibition (mm) of extracts against different test organisms

Plant Extracts	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Salmonella typhimurium</i>
C	15.5±0.71 ^c	16.5±0.71 ^c	18.5±0.71 ^d	10.0±0.0 ^b
1	0.0±0.0 ^a	10.5±0.71 ^b	13.5±0.71 ^c	15.0±1.41 ^c
2	0.0±0.0 ^a	0.0±0.0 ^a	11.5±0.71 ^b	0.0±0.0 ^a
3	15±1.41 ^c	0.0±0.0 ^a	12.5±2.12 ^{bc}	14.0±0.0 ^c
4	10.0±0.0 ^b	12.0±1.41 ^b	15.0±1.41 ^{cd}	15.5±2.12 ^c
5	12.0±0.0 ^{bc}	0.0±0.0 ^a	10.5±0.71 ^b	13.0±1.41 ^c
6	13.5±2.12 ^c	10.0±0.0 ^b	13.5±0.71 ^c	15.5±0.71 ^c
1&2	0.0±0.0 ^a	0.0±0.0 ^a	11.5±0.71 ^b	11±0 ^b
1&3	0.0±0.0 ^a	10.5±0.71 ^b	10.0±0.0 ^b	16.5±0.71 ^{cd}
1&4	10.0±0.0 ^b	11.0±0.0 ^b	12.5±0.71 ^{bc}	18.0±0.0 ^d
1&5	11.0±0.0 ^b	11.5±0.71 ^b	10.5±0.71 ^b	10.5±0.71 ^b
1&6	11.0±0.0 ^b	10.0±0.0 ^b	12.0±0.0 ^{bc}	12.0±1.41 ^{bc}
2&3	11.5±0.71 ^b	0.0±0.0 ^a	0.0±0.0 ^a	15.5±0.71 ^c
2&4	10.5±0.71 ^b	10.5±0.71 ^b	13.5±0.71 ^c	18.5±0.71 ^d
2&5	0.0±0.0 ^a	0.0±0.0 ^a	10.5±0.71 ^b	0.0±0.0 ^a
2&6	11.5±0.71 ^b	0.0±0.0 ^a	10.5±0.71 ^b	11.0±0.0 ^b
3&4	10.0±0.0 ^b	11.5±0.71 ^b	12.5±0.71 ^{bc}	18.0±0.0 ^d
3&5	10.0±0.0 ^b	10.0±0.0 ^b	10.5±0.71 ^b	15.5±0.71 ^c
3&6	11.0±0.0 ^b	10.0±0.0 ^b	10.5±0.71 ^b	15.0±0.0 ^c
4&5	12.0±0.0 ^{bc}	11.0±0.0 ^b	13.0±0.0 ^c	16.5±0.71 ^{cd}
4&6	11.0±0.0 ^b	10.0±0.0 ^b	12.5±0.71 ^{bc}	15.5±0.71 ^c
5&6	11.5±0.71 ^b	10.5±0.71 ^b	10.5±0.71 ^b	11.5±0.71 ^b

Values are mean ± SD of replicates; abcd=values with different superscript for the same test organism (within the same column) are significantly different.

Extracts: C=Gentamicin control, 1= *Azadirachta indica* leaf ethanolic extract, 2= *Carica papaya* leaf ethanolic extract, 3= *Azadirachta indica* stem bark water extract, 4= *Azadirachta indica* stem bark ethanolic extract, 5= *Carica papaya* leaf water extract and 6= *Azadirachta indica* leaf water extract.

Table 2. Minimum inhibition concentration (MIC) (mg /ml) of crude extracts against test organisms

PLANT EXTRACTS	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Salmonella typhimurium</i>
1	-	100 ^a	25 ^c	50 ^b
2	-	-	100 ^a	-
3	100 ^a	-	50 ^b	12.5 ^d
4	100 ^a	100 ^a	12.5 ^d	25 ^c
5	100 ^a	-	50 ^b	50 ^b
6	100 ^a	100 ^a	100 ^a	100 ^a
1&2	-	-	100 ^a	25 ^c
1&3	-	100 ^a	25 ^c	50 ^b
1&4	100 ^a	50 ^b	50 ^b	25 ^c
1&5	100 ^a	100 ^a	100 ^a	100 ^a
1&6	100 ^a	100 ^a	100 ^a	100 ^a
2&3	100 ^a	-	-	100 ^a
2&4	100 ^a	100 ^a	50 ^b	25 ^c
2&5	-	-	100 ^a	-
2&6	100 ^a	-	100 ^a	100 ^a
3&4	100 ^a	100 ^a	50 ^b	25 ^c
3&5	100 ^a	100 ^a	100 ^a	50 ^b
3&6	100 ^a	100 ^a	100 ^a	100 ^a
4&5	100 ^a	100 ^a	50 ^b	25 ^c
4&6	100 ^a	100 ^a	50 ^b	25 ^c
5&6	100 ^a	100 ^a	50 ^b	100 ^a

abcd=values with different superscript for the same test organism (within the same column) are significantly different.

Extracts 1= Azadirachta indica leaf ethanolic extract, 2= Carica papayaleaf ethanolic extract, 3= Azadirachta indica stem bark water extract, 4= Azadirachta indica stem bark ethanolic extract, 5= Carica papaya leaf water extract and 6= Azadirachta indica leaf water extract, -= No activity

Table 3: Phytochemical screening of plant crude extracts

Parameter	Extracts					
	1	2	3	4	5	6
Tannins	+	-	+	+	-	-
Saponins	-	-	+	+	-	-
Flavonoids	+	-	-	+	-	-
Alkaloids	-	-	-	-	-	-
Anthocyanin and Betacyanin	+	+	-	-	+	+
Quinones	+	-	+	+	+	+
Glycoside	-	-	-	-	-	-
Cardiac glycosides	+	-	+	+	-	-
Terpenoids	+	-	+	+	+	+
Phenols	+	+	-	-	+	+
Acids	-	-	-	-	-	-
Triterpenoids	-	-	-	-	-	-

Extracts 1= Azadirachta indica leaf ethanolic extract, 2= Carica papaya leaf ethanolic extract, 3= Azadirachta indica stem bark water extract, 4= Azadirachta indica stem bark ethanolic extract, 5= Carica papaya leaf water extract and 6= Azadirachta indica leaf water extract, -= Absent, += Present.

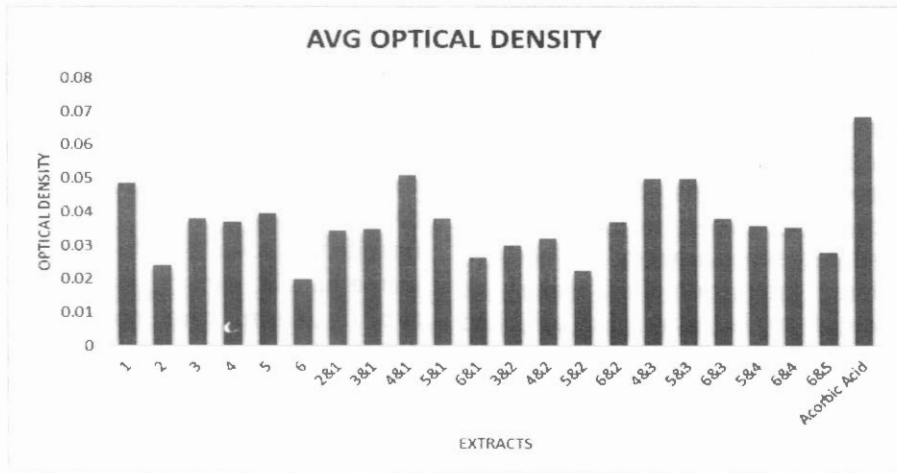


Figure 1: Total Antioxidant capacity assay optical density result

Extracts 1= *Azadirachta indica* leaf ethanolic extract, 2= *Carica papaya* leaf ethanolic extract, 3= *Azadirachta indica* stem bark water extract, 4= *Azadirachta indica* stem bark ethanolic extract, 5= *Carica papaya* leaf water extract and 6= *Azadirachta indica* leaf water extract

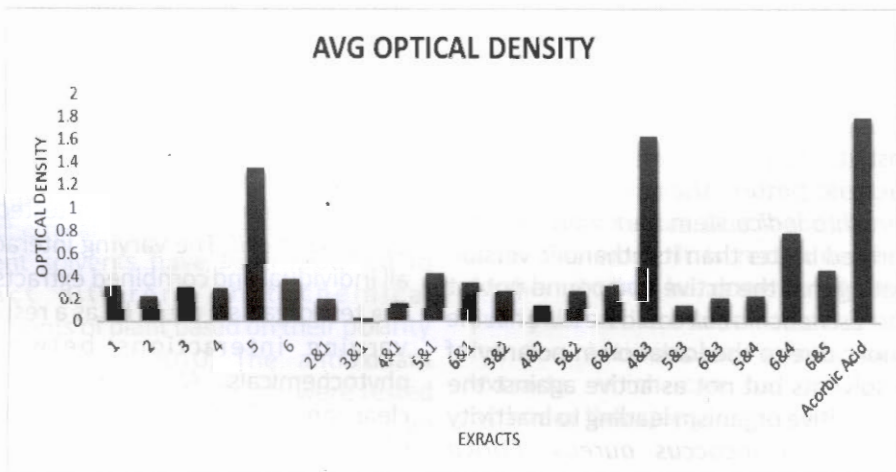


Figure 2: FRAP assay average optical density result

Extracts 1= *Azadirachta indica* leaf ethanolic extract, 2= *Carica papaya* leaf ethanolic extract, 3= *Azadirachta indica* stem bark water extract, 4= *Azadirachta indica* stem bark ethanolic extract, 5= *Carica papaya* leaf water extract and 6= *Azadirachta indica* leaf water extract.

Discussion

Plant extract has great therapeutic potentials against microorganisms and is without undesirable side effect common to synthetic chemotherapeutics. All extracts examined expressed antimicrobial and antioxidant properties. The individual extract and their combinations demonstrated either a synergistic, additive or indifferent interaction effect against the test bacteria while some also exhibited antagonistic effects in line with the report by Ncube et al. (2010). *Azadirachta indica* stem bark ethanolic extract and *Azadirachta indica* leaf water extract were effective against all four test organisms used for this work, *Carica papaya* leaf water extract and *Azadirachta indica* stem bark water extract were potent against all the test organisms except for *Staphylococcus aureus* while *Azadirachta indica* leaf ethanolic extract was potent against all except *Escherichia coli* but *Carica papaya* leaf ethanol extract was only potent against *Bacillus subtilis*. All extracts had clear zones of inhibition against *Bacillus subtilis* and the control (Gentamicin) antibiotic had the highest zone of inhibition against it. Despite reports that ethanolic extract is better than water extracts *Azadirachta indica* stem bark water extract performed better than its ethanolic version indicating that the active compound potent against *Escherichia coli* could be lower in the ethanolic due to the variation in polarity of both solvents but not as active against the Gram-positive organism leading to inactivity against *Staphylococcus aureus*. *Carica papaya* leaf water extract also performed better against Gram-negative test organisms while *Carica papaya* leaf ethanol extract didn't have zones of inhibition against the same organisms. *Azadirachta indica* leaf water extract performed better than

Azadirachta indica leaf ethanolic extract against all test organisms and was potent against *Escherichia coli* against which *Azadirachta indica* leaf ethanolic extract had no activity. The combined extracts worked better against *Salmonella typhimurium* compared to the individual extract and the control antibiotic.

Despite the lack of activity of *Carica papaya* leaf ethanolic extract against *Salmonella typhimurium*, the combination of *Azadirachta indica* stem bark ethanolic extract and *Carica papaya* leaf ethanolic extract produced a higher zone of inhibition compared to *Azadirachta indica* stem bark ethanolic extract alone for the same organism confirming the synergistic potentials of some of the extracts when combined. *Azadirachta indica* leaf water extract and *Carica papaya* leaf water extract expressed antagonistic interaction against *Salmonella typhimurium* compared to the performance of the individual extracts resulting in the reduction of the zone of inhibition produced by the extract individually. The combination of *Carica papaya* leaf ethanol extract and *Azadirachta indica* leaf ethanolic extract had an indifferent interaction when tested against *Escherichia coli*. The varying interactions of all individual and combined extracts against the test organism could be as a result of the varying interactions between the phytochemicals. All the extracts expressed clear zones of inhibition against at least one of the bacterial isolates used in this research buttressing the fact that folk herbal drugs have great potential as antimicrobial agents. The highest individual extract inhibition zone was 15.5 ± 2.12 mm and for combined extract was 18.5 ± 0.71 mm against *Salmonella typhimurium*. In the analysis for

the MIC of extracts that have activity against the test organism, most extracts and combinations became inactive against the test organisms at 100mg/ml agar dilution. *Azadirachta indica* stem bark ethanolic extract and *Azadirachta indica* stem bark water extract were not effective against the test organisms' colony formation at 12.5mg/ml concentration. Most of the extracts had a better result against *Salmonella typhimurium*. All extracts had antioxidant activity and some of the phytochemicals present in the extracts include saponins, flavonoids, tannins, anthocyanin, betacyanin, quinones, cardiac glycoside, terpenoids, and phenols. The phytochemical analysis as reported in Table 3 showed that the phytochemicals results of *Azadirachta indica* leaf and stem bark extract is in line with earlier reports where water and ethanol extracts of the same plant material were extracted, the ethanol solvent have been reported to perform better than water in extraction. Hence, contrary to the observations in the *Azadirachta indica* plant extracts in this study, the phytochemical screening of *Carica papaya* leaf extract showed more phytochemical content in the water extract than in the ethanolic extract, this could be due to the polarity of the phytochemical component of the plant, different solvents have been reported to extract different phytochemical constituents of plant based on their polarity (Suleiman et al.,2010) The antioxidant potentials of the plant extracts were tested in comparison to ascorbic acid which is an established antioxidant and the result of the FRAP assay showed that all the extracts had antioxidant potentials with *Azadirachta indica* stem bark ethanolic extract and *Azadirachta indica* stem bark water extract having the highest activity and *Azadirachta*

indica stem bark water extract and *Azadirachta indica* leaf ethanolic extract with the lowest activity. The total antioxidant activity assay also showed significant antioxidant potentials in comparison to ascorbic acid which is the positive control. *Azadirachta indica* leaf ethanol extract, *Azadirachta indica* stem bark ethanol extract and *Azadirachta indica* leaf ethanol extract, *Azadirachta indica* stem bark ethanol extract and *Azadirachta indica* stem bark water extract and *Carica papaya* leaf water extract and *Azadirachta indica* stem bark water extract had the most prominent values in comparison to ascorbic acid while *Azadirachta indica* leaf water extract had the least.

Conclusion

This study shows the synergistic therapeutic potentials of *Carica papaya* and *Azadirachta indica* leaves and stems bark ethanolic and aqueous extracts in vitro against some clinically important Gram-positive and Gram-negative strains of bacteria. The synergistic effect from some of the combined plant extract could be considered as sources of potential biotherapeutic agents that would be less toxic to patients and more potent against infectious diseases. Due to the observation from this work, it is recommended that further studies be done to identify and purify the active biomolecules responsible for the antimicrobial effects of these extracts which can be employed for orthodox therapies.

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References

- Akula, C., Akula, A., & Drew, R. (2003). Somatic embryogenesis in clonal neem, *Azadirachta indica* A. Juss. and analysis for in vitro azadirachtin production. *In Vitro Cellular & Developmental Biology-Plant*, **39** (3), 304–310. <https://doi.org/10.1079/IVP2003415>
- Alabi, O. A., Haruna, M. T., Anokwuru, C. P., Jegede, T., Abia, H., Okegbe, V. U., & Esan, B. E. (2012). Comparative studies on antimicrobial properties of extracts of fresh and dried leaves of *Carica papaya* (L) on clinical bacterial and fungal isolates. *Pelagia Research Library Advances in Applied Science Research*, **3** (5), 3107–3114. Retrieved from www.pelagiaresearchlibrary.com
- Alviano, D. S., & Alviano, C. S. (2009). Plant extracts: search for new alternatives to treat microbial diseases. *Current Pharmaceutical Biotechnology*, **10** (1), 106–121. <https://doi.org/10.2174/138920109787048607>
- Ang, J. Y., Ezike, E., & Asmar, B. I. (2004). Antibacterial Resistance. *Indian Journal of Pediatrics*, **71**(3), 229–239. <https://doi.org/10.1007/BF02724275>
- Balouiri, M., Sadiki, M., & Ibensouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, **6** (2), 71–79. <https://doi.org/10.1016/j.jpha.2015.11.005>
- Boucher, H. W., Talbot, G. H., Bradley, J. S., Edwards, J. E., Gilbert, D., Rice, L. B., ... Bartlett, J. (2009). Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, **48**(1), 1–12. <https://doi.org/10.1086/595011>
- Chanda, S., & Dave, R. (2009). In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *African Journal of Microbiology Research*, **3**(13), 981–996. <https://doi.org/10.1007/s13197-011-0276-5>
- Chin, Y. W., Balunas, M. J., Chai, H. B., & Kinghorn, A. D. (2006). Drug discovery from natural sources. *AAPS J*, **8** (2), E 239 - 53. <https://doi.org/10.1208/aapsj080228>
- Crentsil, K. B., Archibold, B.-K., & Jacob, A. (2011). Morphological studies of Neem (*Azadirachta indica* A. Juss.) seed and physicochemical properties of its oil extracts collected in Accra metropolis of Ghana. *Elixir International Journal*, **2011** (39), 4951–4953. http://www.elixirpublishers.com/articles/1350636834_39 (2011) 4951-4953.pdf
- El-Mahmood, A. M., Ogbonna, O. B., & Raji, M. (2010). The antibacterial activity of *Azadirachta indica* (neem) seed extracts against bacterial pathogens associated with eye and ear infections. *Journal of Medicinal Plants Research*, **4** (14), 1414–1421. <https://doi.org/10.5897/JMPRO9.169>
- El Moussaoui, A., Nijs, M., Paul, C., Wintjens, R., Vincentelli, J., Azarkan,

M., & Looze, Y. (2001). Revisiting the enzymes stored in the laticifers of *Carica papaya* in the context of their possible participation in the plant defence mechanism. *Cellular and Molecular Life Sciences*, **58**(4), 556 – 570. <https://doi.org/10.1007/PL00000881>

Hashmat, I., Azad, H., & Ahmed, A. (2012). Neem (*Azadirachta indica* A. Juss) - A Nature TMs Drugstore: An overview. *International Research Journal of Biological Sciences*, **1**(6), 76–79. Retrieved from <http://www.isca.in/IJBS/Archive/v1/i6/14.ISCA-IRJBS-2012-150.php>

Kamba, A. S., & Hassan, L. G. (2010). Phytochemical screening and antimicrobial activities of *Euphorbia balsamifera* leaves, stems and root against some pathogenic microorganisms. *African Journal of Pharmacy and Pharmacology*, **4** (9), 645–652. Retrieved from <http://www.academicjournals.org/ajpp>

Kaura, S. K., Gupta, S. K., & Chowdhury, J. B. (1998). Morphological and oil content variation in seeds of *Azadirachta indica* A. Juss. (Neem) from northern and western provenances of India. *Plant Foods for Human Nutrition (Dordrecht, Netherlands)*, **52** (4), 293–298. <https://doi.org/10.1023/A:1008013424150>

Li, J., Lin, X., Zhang, Y., Liu, W., Mi, X., Zhang, J., & Su, J. (2016). Preparative Purification of Bioactive Compounds from *Flos Chrysanthemi Indici* and Evaluation of

Its Antiosteoporosis Effect. *Evidence-Based Complementary and Alternative Medicine*, 2016, 1–12. <https://doi.org/10.1155/2016/2587201>

Mello, V. J., Gomes, M. T. R., Lemos, F. O., Delfino, J. L., Andrade, S. P., Lopes, M. T. P., & Salas, C. E. (2008). The gastric ulcer protective and healing role of cysteine proteinases from *Carica candamarcensis*. *Phytomedicine*, **15** (4), 237 – 244. <https://doi.org/10.1016/j.phymed.2007.06.004>

Milugo, T. K., Omosa, L. K., Ochanda, J. O., Owuor, B. O., Wamunyokoli, F. A., Oyugi, J. O., & Ochieng, J. W. (2013). Antagonistic effect of alkaloids and saponins on bioactivity in the quinine tree (*Rauvolfia caffra* sond.): further evidence to support biotechnology in traditional medicinal plants. *BMC Complementary and Alternative Medicine*, **13** (1), 285. <https://doi.org/10.1186/1472-6882-13-285>

Mohammed, H. A., Fadhil, A., & Omer, A. (2015). Antibacterial Activity of *Azadirachta indica* (Neem) Leaf Extract against Bacterial Pathogens in Sudan. *American Journal of Research Communication*, **3** (5), 246–251.

Nascimento, G. G. F., Locatelli, J., Freitas, P. C., & Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, **31** (4), 247–256. <https://doi.org/10.1590/S1517-83822000000400003>

- Njimoh, D. L., Assob, J. C. N., Mokake, S. E., Nyhalah, D. J., Yinda, C. K., & Sandjon, B. (2015). Antimicrobial Activities of a Plethora of Medicinal Plant Extracts and Hydrolates against Human Pathogens and Their Potential to Reverse Antibiotic Resistance. *International Journal of Microbiology*, 2015, 547156. <https://doi.org/10.1155/2015/547156>
- Orhue, P. O., Momoh, A. R. M., Igumbor, E. O., & Esumeh, F. (2014). Antibacterial effect of *Azadirachta indica* (CN : Neem or Dongo Yaro) parts of some urinary tract bacterial isolates. *Asian Journal of Plant Science and Research*, 4 (2), 64–67. www.pelagiaresearchlibrary.com
- Sukanya, S., Sudisha, J., Hariprasad, P., Niranjana, S., Prakash, H., & Fathima, S. (2009). Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. *African Journal of Biotechnology*, 8 (23), 6677 – 6682 . <https://doi.org/10.4314/ajb.v8i23.66376>
- Suleiman, M. M., McGaw, L. J., Naidoo, V., & Eloff, J. N. (2010). Detection of antimicrobial compounds by bioautography of different extracts of leaves of selected South African tree species. *African Journal of Traditional, Complementary and Alternative Medicines*, 7 (1), 64–78. <https://doi.org/10.4314/ajtcam.v7i1.57269>
- Suleiman, M. N. (2011). The in vitro phytochemical investigation on five medicinal plants in Anyigba and its environs, Kogi State, Nigeria. *Pelagia Research Library*, 2 (4), 108–111. Retrieved from <http://www.imedpub.com/articles/the-in-vitro-phytochemical-investigation-on-five-medicinal-plants-in-anyigba-and-its-environs-kogi-state-nigeria.pdf>
- Wiegand, I., Hilpert, K., & Hancock, R. E. W. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, 3(2), 163–175. <https://doi.org/10.1038/nprot.2007.521>