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Phytochemical Screening and Antimicrobial Activity of Leaves Extracts of *Mangifera indica* and *Carica papaya*

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ABSTRACT

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Phytochemical and antimicrobial screening analysis of *Mangifera indica* and *Carica papaya* indicated the presence of alkaloid, saponin, flavonoid, steroid, tannin, phenol and glycoside. Extract of *Mangifera indica* and *Carica papaya* were investigated for their antimicrobial activities. The ethanolic extract produced zone of inhibition higher in diameter than aqueous extract against *Escherichia coli*, *Pseudomonas spp*, *Staphylococcus spp*, *Streptococcus spp* and *salmonella spp*.

Introduction

It is a known fact that in order for a plant to protect itself, it produces some chemicals which can also protect humans against diseases. As many as over 4000 different phytochemicals having potential to affect diseases such as cancer, stroke or metabolic system are in existence today (Arts and Hollman, 2005). Phytochemicals are chemical compounds that naturally occur in plants which are responsible for many attributes of the plants such as smell, taste, colour and other organoleptic properties of the plant. They are classified into curative (or nutritive) such as alkaloid, saponins, tannins, flavonoids, phenols, glycosides, isoflavones, cartenoids, sulfides, and non-curative (or non-nutritive) such as cyanide, oxalates, terpenes,

terpenoids. Though some phytochemicals may have their biological significance because of their established essential nutritional values, however many have been considered as potential drugs because of their therapeutic potentials (Levin *et al.*, 1979). Plants with such phytochemicals are regarded as medicinal plant because they have similar properties as conventional pharmaceutical drugs. The local use of natural plants as primary health remedies, due to their pharmacological properties is quite common. In many part of the world especially in West Africa plant extracts are still widely used in the treatment of malaria and other ailments (World Health Organization, 2002). As a matter of fact, every plant is medicinal on the

basis of the contents of their phytochemicals, hence pharmaceutical industries rely heavily on their therapeutic purpose so as to be used as precursors for drug synthesis (Bouayed *et al.*, 2007).

This is because every plant has medicinal value based on the active compounds that can be extracted from its part such as leaves, stems, barks, roots, bulks, rhizomes, woods, flowers, fruits or the seeds for therapeutic purposes. Most phytochemicals such as sulfides (in onions, leeks, and garlic), carotenoid (in carrots), flavonoids (in fruits, vegetables), polyphenols (in tea, grapes) have antioxidant activity and protect our cells oxidative damage and reduce the risk of developing certain types of cancer. Some exhibit hormonal actions such as isoflavones (in soy) which imitate human estrogen and help to reduce menopausal symptoms. Some also stimulate enzyme such as indoles (in cabbages) which makes the estrogen less effective and reduces the risk of breast cancer. Caspacin (in hot pepper) protects DNA from carcinogens while allicin (in garlic) has anti bacteria properties (Manach, 2004).

Alkaloids are naturally occurring organic bases which contain a pyridine ring, invoke a bitter taste and are used in making anti-malaria, hypertension and anti-cancer drugs (Manske, 2009; Kittakoop *et al.*, 2014). Flavonoids have 15-carbon skeleton which consists of two phenyl ring and heterocyclic ring and are known for their anti-inflammatory and anti-allergic effects (Yamamoto and Gaynor, 2001). Glycosides are molecules in which sugars are bound to another functional group via a glycosidic bond (either O- or S-). Digitalis glycosides have served as cardiac drugs in case of heart failure (Brito-Arias and Marco, 2007). Phenols are a class of aromatic organic compound which possess antiseptic properties and may be used as disinfectant (Amorati and

Valgimidi, (2012). Saponin are plant glycosides which serve as anti-feedant and protects plants against microbes and fungi. Some plant saponin aids in nutrient absorption and digestion (Francis, *et al.*, 2002). Steroids are polycyclic compounds which have alkanol functional group and they include cholesterol, the sex hormones (Desmond and Gribaldo, 2009). Tannin refers are phenolic compounds characterized by their ability to precipitate proteins, amino acids and alkaloids. They protect the plant from predation perhaps as pesticides and regulate its growth and helps in the ripening of fruits (Drabble and Nierenstein, 2001).

An antimicrobial is an agent either kills microorganisms or inhibits their growth but causes little or no damage to the host. Antimicrobial therapy refers to the use of antimicrobial medicines to treat infection while antimicrobial prophylaxis refers to the use of antimicrobial medicines to prevent infection (Amyes 1996).

In formulation of animal feed, plant materials are used because they contain phytochemicals which can serve as antibiotic to the animals. Hence there is need to investigate common plants which are easily available, cheap, renewable and nutritive source of material as feed supplements. *Mangifera indica* and *Carica papaya* being edible they have been reported along with the roots and leaves to be of medicinal value (Fowomola, 2010). *Mangifera indica* also known as mango whose chemical constituent include pharmacologically active hydroxylated xanthone C-glycoside which is extracted from the leaves and bark (Jonathan, 1993) and allergenic urushiols which is extracted from the fruit peel (Cuadra, Pablo, 2007). These phytochemicals are useful as anti-diuretic, anti-diarrheal, anti-emetic and cardiac herb (Gordon, 2012). On the other hand, *Carica papaya* and is one of species in the genus

Carica of the plant family *Caricaceae*. Papaya leaves are made into tea Fowomola, as a treatment for malaria (Titanji, *et al.*, 2008).

The major aim of this work therefore was to determine the phytochemicals and antimicrobial agents present in *Mangifera indica* and *Carica papaya*.

The phytochemical analysis are carried out first qualitatively and then quantitative (Nijveldt, 2001).

Materials and Methods

Collection and identification of samples

Sample of *mangifera indica* and *carica papaya* were obtained on 19th September 2016, from Ama-Oba village, Afikpo South and they were authenticated in the Department of Applied Biology, Ebonyi State University, Abakaliki, Ebonyi, Nigeria.

Preparation of samples

Freshly collected leaves of *mangifera indica* and *carica papaya* were cleaned and sun-dried before they were pulverized using mortar and pestle into smaller particles after which they were blended to powder using an electric blender. The powdered samples were stored in airtight containers and kept under normal room temperature until required.

Preparation of aqueous extracts

50grams of the dried powdered samples were weighed and soaked in 250ml of distilled water contained in two different 250ml flasks and stirred for five minutes. The flask were covered with foil and then allowed to stand for 48 hours. After 48 hours, the suspensions were shaken vigorously and filtered using filter paper.

Preparation of ethanolic extracts

50grams of the dried powdered samples were weighed and soaked in 250ml of ethanol contained in two different 250ml flasks and stirred for five minutes. The flasks were covered with foil and then allowed to stand for 48 hours. After 48 hours, the suspensions were shaken vigorously and filtered using filter paper.

Preparation of Mayer's reagent

1.3g of mercuric chloride and 5.0g of potassium iodide were dissolved in distilled water in a 100ml volumetric flask and the solution was made up to 100ml

Antimicrobial susceptibility procedure

Five organisms were used in this study, consisting of three gram negative (*Salmonella*, *Escherichia coli* and *Pseudomonas Spp*) while the other two were gram positive (*Staphylococcus* and *Streptococcus Spp*). All the bacteria were isolated from microbiology laboratory unit of Ebonyi State University, Abakaliki.

Antimicrobial assay

This was done using protocol described by Owosemi and Ajayi (2010). The test bacteria were sub-cultured onto fresh peptone water medium. Broth cultures were then incubated at 37°C till the turbidity of 0.5 McFarland standards was obtained. The turbidity of the actively growing broth culture was then adjusted with sterile water to obtain 0.5 McFarland turbidity standards. This was streaked on the surface of solid Mueller Hinton agar plates using sterile cotton swab stick. Wells of 8mm in diameter and about 2cm apart were punched in the culture media with sterile cork borer; the extracts were thereafter used to fill the boreholes. The plates

were incubated at 37°C for 24 hours. Zones of inhibitions around the wells, measured in millimetres were recorded.

Phytochemical screening

Testing for alkaloid

3ml of the extract was pipette into a test tube and to the extract was added 1ml of 1% HCl, it was heated for twenty minutes in a water bath and then allowed to cool. After cooling 0.5ml of Mayer's reagent was added. The appearance of creamy white color indicates the presence of alkaloid.

Test for flavonoid

3mls of the extract was pipette into a test tube to it was added 10mls of distilled water, and then to it was added 10mls of distilled water, then 1ml of 10% NaOH. The change in the color of the mixture to yellow indicates a positive.

Test for glycosides

3ml of the extract was pipette into test tubes to it was added 1ml of 2% 3, 5-dinitrosalicylic acid in methanol and 1ml of 5% NaOH a change in the color of the mixture to orange indicates the presence of glycoside.

Test for phenols

1ml of the extract was pipette into a test tube, to it was added 1ml of distilled water and 3-4 drops of 5% NaOH. An orange coloration indicates the presence of phenol

Test for saponin: (Frothing test)

3ml of the extract was pipette into a test tube, to it was added 2ml of distilled water, it was shook vigorously, and a persisting frothing movement indicates the presence of saponin

Test for steroids

1ml of the extract was pipette into a test tube 5 drops of concentrated H₂SO₄ was added a red coloration indicate the presence of steroids.

Test for tannins

2mls of the extract was pipette into a test tube and was boiled in a water bath for twenty minutes, after cooling 3 drops of 1% ferric chloride was added to it; a bluish precipitate indicates the presence of tannin.

Results and Discussion

Table 1 presents Qualitative Phytochemical Analysis of *M. indica* and *C. papaya* leaves; Table 2 presents Antimicrobial activities of water extract of *Mangifera indica* and *Carica papaya* leave against test organisms. Zone of inhibition diameter and table 3 presents Antimicrobial activities of ethanol extract of *Mangifera indica* and *Carica papaya* leave against test organisms.

Table 1 revealed the presence of saponin, flavonoid, steroid, tannin and glycoside in varying qualities in the ethanol and water extracts. Saponin in ethanol extract and flavonoid in water extract of *mangifera indica* had the strongest positive presence.

On the other hand, steroid was not detected in water extract of *mangifera indica* and ethanol extract of *carica papaya*. In addition, phenol and glucoside were not detected in ethanol extracts of *Mangifera indica* and *Carica papaya* respectively. All the phytochemicals that were detected are known to have industrial and medicinal importance. For example, saponin is useful in medicine as antioxidant, anticancer and for treatment of hypercholesterolemia and hyper glycaemia. It is also used as a mild detergent and in

intracellular histochemistry staining to allow antibody access to intracellular protein. Tannin exhibits antiviral, antibacterial, antitumor activities. It was also reported that certain tannins are able to inhibit HIV replication selectivity (Drabble and Nierenstein, 2000). Steroid play important role in cardio tonic activities, they possess insecticidal and antimicrobial properties. They are very useful in the nutrition, herbal medicine and cosmetics industries. They are

routinely used in medicine because of their profound biological activities. Flavonoid has inherent ability to modify the body's reaction to allergies, virus and carcinogens (Erdman *et al.*, 2007). They show anti allergic, anti-inflammatory, antimicrobial and anticancer activities. Alkaloid has the potency to correct serious disorders such as heart failure, cancer and blood pressure. Glycosides are known to work by inhibiting the Na⁺ / K⁺ pump (Manske, 2009).

Table.1 Qualitative Phytochemical Analysis of *M. indica* and *C. papaya* Leaves

PHYTOCHEMICAL PARAMETERS	MANGIFERA INDICA		CARICA PAPAYA	
	WATER EXTRACT	ETHANOL EXTRACT	WATER EXTRACT	ETHANOL EXTRACT
Alkaloid	++	+	+	++
Saponin	+	+++	++	+
Flavonoid	+++	+	++	++
Steroid	-	++	+	-
Tannin	+	+	+	+
Phenol	++	-	+	+
Glycoside	+	+	++	-

+++ = strongly positive, ++ = positive, + = fairly positive, - = not detected

Table.2 Antimicrobial Activities of Water Extract of *Mangifera indica* and *Carica papaya* Leaves against Test Organisms

ORGANISMS	DIAMETER OF ZONE OF INHIBITION OF WATER EXTRACT									
	<i>Mangifera indica</i>					<i>Carica papaya</i>				
	1	2	3	4	5	1	2	3	4	5
<i>Escherichia coli</i>	22	20	15	12	8	17	14	12	17	14
<i>Pseudomonas spp</i>	22	20	17	16	12	17	12	17	16	14
<i>Staphylococcus spp</i>	23	20	17	17	15	17	16	14	12	16
<i>Streptococcus spp</i>	18	15	12	13	14	19	16	13	12	9
<i>Salmonella spp</i>	20	17	14	13	10	18	15	11	12	9

Table.3 Antimicrobial Activities of Ethanol Extract of *Mangifera indica* and *Carica papaya* Leaves against Test Organisms

ORGANISMS	DIAMETER OF ZONE OF INHIBITION OF ETHANOL EXTRACT									
	<i>Mangifera indica</i>					<i>Carica papaya</i>				
	1	2	3	4	5	1	2	3	4	5
<i>Escherichia coli</i>	28	24	20	17	15	25	21	17	21	20
<i>Pseudomonas spp</i>	28	23	22	20	17	24	20	21	20	20
<i>Staphylococcus spp</i>	31	26	23	23	22	25	22	20	18	24
<i>Streptococcus spp</i>	25	20	17	20	21	27	22	18	21	18
<i>Salmonella spp</i>	26	24	21	20	20	23	21	15	16	19

Tables 2 and 3 showed low activity with minimum diameter inhibition zone of 8mm for *E. coli* and maximum 23mm for *staphylococcus*, *streptococcus* and *salmonella*. Whereas the ethanol extract showed higher activity against with minimum diameter inhibition zone of 15mm for *E. coli* and maximum 31mm for *staphylococcus*. The difference in the observed activities of the various extracts could be as a result of varying degree of solubility of the active constituents in the two solvents used. Different solvents are known to have different solubility capacities for different phyto constituents (Marjorie, 1999). The difference in activities among the solvents recorded in this study could also be affected by the presence of oil, wax, resins, fatty acid or pigments, which all have the capacity to block the active ingredient in the plant extract; thus, preventing the plant extract from accessing the bacteria cell wall (Jigna *et al.*, 2006).

From the results, ethanolic extract of *M. indica* demonstrated a higher activity with respect to the different zones. For *staphylococcus* at zone 1, 2, 3, 4 and 5; zones in diameter were 31mm, 26mm, 23mm, 23mm and 22mm. These results agree with those reported by El-Mahmood *et al.*, (2008). The crude extract can further from ethanolic extraction can further be refined into pure form and use it against pathogens that cause infection in local communities Tannin, saponin, steroid, flavonoid and glycoside are major phytochemicals that contribute to the inhibitions (Fowomola, 2012).

Based on the obtained results, leaves of *Mangifera indica* and *Carica papaya* contains some potential phytochemicals that exhibit antimicrobial activity with ethanolic extract being the most potent than water extract. Apparently this finding is in tandem with the claim by the local communities for its potential use as therapeutic agent for the

treatment of urinary tract infection, respiratory infection and stomach pain.

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