

Qualitative Phytochemical Analysis and in Vitro Fungal Activities of *Citrus aurantifolia* L. and *Carica papaya* L. Leaves Extracts on Pathogenic Fungi

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Abstract: The use of medicinal plants, which have fewer side effects and economically cheaper, have been taken into consideration recently. *Citrus aurantifolia* and *Carica papaya* leaves are not only known for their nutritional benefits but also considered to possess medicinal properties. The main aim of the study was to determine the antifungal activities of *Citrus aurantifolia* and *Carica papaya* leaves on some pathogenic fungi (*Aspergillus*, *Candida* and *Penicillium*) isolated from female genital tract. Leaves of *Citrus aurantifolia* and *Carica papaya* were collected from Onu-Ebonyi in Ebonyi State. Three different concentrations (300 mg/ml, 150 mg/ml and 75 mg/ml) of leaves ethanol extract were prepared and introduced into bored holes of organisms cultured on potato dextrose agar (PDA). The result showed that leaf extract of lime had higher zones of inhibition diameter on three fungi species compare to leaf ethanol extract of *Carica papaya*. There were increase on zones of inhibition of both leaves extract as the concentration increases from 75 mg/ml to 300 mg/ml. Phytochemical screening indicated the presence of flavonoids, alkanoids, tannins, saponin and protein in *Carica papaya* but alkanoids and tannins in *Citrus aurantifolia*. The result showed that *Citrus aurantifolia* and *Carica papaya* possess antifungal activities but *Citrus aurantifolia* showed more inhibitions on *Aspergillus*, *Candida* and *Penicillium* than *Carica papaya*.

Keywords: Fungi, Inhibition, *Citrus aurantifolia*, *Carica papaya*, Isolates

I. Introduction

The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbours an inexhaustible source of active ingredients valuable in the management of many diseases. Most plants have medicinal values and the active components are normally extracted from all plant structures; the leaves, stems, barks, roots, corms, rhizomes, woods, flowers, fruits or the seeds, however the concentrations of these components vary from structure to structure. The parts known to contain the highest concentration of chemical constituents are preferred for therapeutic purposes (Kafaru, 1994).

Some of the active principles singly or in combination inhibit greatly the life processes of microbes, especially the disease causing ones. They do this by binding their protein molecules, acting as chelating agents (selective binding polyvalent metal ions so that the latter loses its biological activities), altering their biochemical systems, preventing utilization of available interests to the microorganisms, others cause inflammation of microbial cells (Garrod *et al.*, 1995). The bitter taste, pungent and repulsive smell in some plants; have been found to have repressive ability over the metabolic activities of a wide range of microorganisms (Mitscher *et al.*, 1992; Sofowora, 1982; Baladrin *et al.*, 1985).

The use of medicinal plants predates the introduction of antibiotics and other modern drugs into the African continent. The active components plants are expected to be inimical to the growth of at least some microorganisms especially the disease causing ones e.g. *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* etc. therefore, many studies and researches had been done on the antimicrobial properties of many plants (Okafor, 1991).

Carica papaya is a herbaceous fruit crop belonging to the family Caricaceae, pawpaw is the fruit of the plant *Carica papaya*, the only species in the genus *Carica* of the plant family Caricaceae. It is native to the tropics of the Americas, mostly grown in tropical and subtropical regions (Aravind *et al.*, 2013). It is distributed through out Asia, Nigeria etc. (Afolayan, 2003). The ripe fruit of papaya is usually eaten raw, without the skin or seeds but the unripe green fruit can be eaten cooked, usually in curries, salads and stews (Okafor, 1998). The plant is also described in a documented property forms and it act as analgesic, amebicide, antibacterial, cardi tonic, cholagogue, digestive, emenagogue, febrifuge, hypotensive, laxative, pectoral, stomachic and vermifuge. It is also a rich source of three powerful antioxidant, vitamin C, vitamin A and vitamin E; the minerals; magnesium and potassium, it contains B vitamin, pantothenic acid, folate and fiber. In addition to all this, it contains a digestive enzyme -papaintha which effectively treats causes of trauma, allergies and sports injuries (Aravind *et al.*, 2013). The edible part of papaya is widely used all over the world. The unripe fruit of

papaya is used as mild laxative and abortifacient agent and leaves are used for treatment of pyrexia, diabetes, gonorrhoea, syphilis, inflammation and as a dressing component for wounds (Gill,1992)

Many biologically active phytochemical have been isolated from papaya and studied for application, almost all parts of the papaya (leaves, latex, seed, fruit, bank, peel, roots) have important biologically active substances that can be isolated for application predominantly in the pharmaceutical industry. Different parts of the plant are attributed with different medicinal values, for example, the seed are effective as a vermifuge and in the treatment of hypertension, diabetes mellitus and hyper cholesterolemia. The fresh leaves are also efficacious in the treatment of gonorrhoea, syphilis and amoebic dysentery. Results from studies on biological activities of *C. papaya* plants extracts and isolated compounds showed that the latex and root extracts inhibited *Candida albicans* (Flath and Forrey, 2007).

Citrus aurantifolia (lime) belongs to family Rutaceae, the fruit is green in colour and oval or round shape with a diameter between one to two inches. Limes can either be sour or sweet, sour limes possess a greater sugar and citric acid content than lemons and feature an acidic and tart taste, while sweet limes lack citric acid content and are sweet in flavor (Loizzo *et al.*, 2012). It is an essential ingredient in the preparation of most herbal concoction; it is also used to suppress stomach ache. When added to sugar and palm oil or to honey, the juice has been found to be an excellent cough relieving mixture. The rind is burnt in some homes to act as insecticides against mosquitoes (Aibinu *et al.*, 2007). There is limited studies on the medicinal value of lime leaf

The aim of the study is to determine the antifungal activities of pawpaw and lime leaves on fungi (*Penicillium*; *Aspergillus* and *Candida*) isolated from female genital tract.

II. Materials and Methods

Collection of Specimen

Permission was obtained from the hospital and patients consent was sort prior to sampling. Each female was asked to recline on the examination bed and open legs wide. A sterile swab was inserted into the vaginal wall, rotated for few seconds before it was withdrawn. The swab sticks were then put back into the container, represented with alphabets, specimen were transported to the laboratory immediately, and stored in the refrigerator prior to medium preparation. Potato dextrose agar media were prepared, an inoculum pool was made using distilled water with swab specimen to get a homogenized mixture. Then a sterile syringe was used to pipette 1 ml of the mixture to spread on the plates. The most preponderant fungi (*Candida*, *Aspergillus* and *Penicillium*) were selected for antifungal studies using *Carica Papaya* and *Citrus aurantifolia*

Sample Collection and Preparation

Fresh leaf samples of *Carica papaya* and *Citrus aurantifolia* were collected from Onu-Ebonyi in Ebonyi State and were transported to Applied Biology Laboratory. The leaves were thoroughly washed with sterile distilled water, and air dried at 28 °C for 2 hours. Both plant samples were dried in an oven at 45 °C for 30 minutes and then pulverized separately with a household blender. The crude extracts of the two plants were prepared using standard procedures (Fatope *et al.*, 1999). This involved soaking 50 g of the powdered extract in 5 % ethanol for 48 hrs at room temperature to allow for maximum extraction of the components. This was followed by evaporation of the filtrate to eliminate the solvent using a rotary evaporator. The residue was used as crude extract for each of the test plants and stored in reagent bottles in the refrigerator at 10- 12 °C until they were used. Three different concentrations of extract of *Carica papaya* and *Citrus aurantifolia* leaves (300 mg/ml, 150 mg/ml and 75 mg/ml) and 5 % ethanol as control were prepared, 2 ml of extracts and control were introduced into the bored holes of pure cultures of organisms. Inhibition zones were measures with meter rule (cm)

Qualitative Phytochemical Screening

The chemical test for the qualitative analysis of *Carica papaya* and *Citrus aurantifolia* leaves were carried out using standard method.

III. Result And Discussion

Citrus aurantifolia leaves extract exhibited higher zones of inhibitions diameter compared to *Carica papaya* and control (5 % ethanol) on three pathogenic fungi. There was an increase on zones of inhibition on *Aspergillus* as the concentration of the extract increases from to 75 mg/ml (1.0 cm), 150 mg/ml (2.2 cm) to 300 mg ml (3.1 cm). Also higher increase in inhibition diameters were recorded on *Candida* and *Penicillium* as the leaf extract concentration increases from 75 mg/ml(1.0 cm), 150 mg ml (2.0 cm) to 300 mg/ml (3.5 cm) and 75 mg/ml(1.2 cm), 150 mg/ml (1.5 cm) to 300 mg/ml (3.2 cm) respectively(Table 1).

Anigboro *et al.*, (2011) reported that extracts of *Citrus aurantifolia* and *C. papaya* leaves showed a better antibacterial activity than antifungal activity. They found the zones of inhibition on the fungi to be the

least of the various zones of inhibition recorded in their study. This therefore suggests that *Citrus aurantifolia* and *Carica papaya* could be better used for the treatment of bacteria infections than for the treatment of fungi infections. Though the efficacy of treatments with *Citrus aurantifolia* and *C. papaya* is dependent on the quantity of the different chemical substances present in their leaf extracts.

In regards to phytochemical analysis, *Carica papaya* leaves showed presence of flavonoids, alkanoids, saponin, tannin and protein. Flavonoid, saponin and protein were absent in leaf extract of *Citrus aurantifolia* (Table 2). However tannins, alkanoids and other unidentified chemical constituents in *Citrus aurantifolia* possess a more biocide activity on fungi isolates than the constituents of *Carica papaya*. The leaves of many plants possess tannins in an abundant quantity and may have biocide activity. According to Canini (2007), the main compounds contained by *C. papaya* leaves are phenolic acids, as well as trace amounts of chlorogenic acid, compared to flavonoids and coumarin compounds. In their study, the authors suggested that the presence of such phenolic and coumarin compounds in *C. papaya* leaves could partially explain the pharmacological properties of this plant.. The quantity of these chemical substances could vary in their fruit, leaves, and roots and could also depend on extraction method, age of the plant part and the cultivar of the tree.

Arumugam *et al.* (2014) indicated the following phytochemicals in *Carica papaya* leaves; protein, flavonoids, saponins, glycoside, steroids, terpinoides, alkaloids, coumarins, phenol, tannins, anthrocyanin and leucoanthocyanin. Doughari *et al.*, 2007 identified saponin, alkaloids, tannins, phenoid in methanoic extract of *Carica papaya* root. Cowan, 1999 showed that extracts from different papaya tissues are bioactive, the leaves and seeds containing proteolytic enzymes (papain, chymopapain), alkaloids (carpain, carpasemine), sulfurous compounds (benzyl isothiocyanate), flavonoids, triterpenes, organic acids and oils.

Mangalanayaki and Nirosha, 2013 examined the antibacterial activity of the leaves of the *Carica papaya* using solvents ethanol and ethyl acetate against *Staphylococcus aureus*, *Streptococcus pneumonia*, *Bacillus cereus*, *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa* by well diffusion method. The extract demonstrated higher activities against all the Gram negative bacteria than Gram positive bacteria tested, with the highest activity (16 mm zone of inhibition) against *Salmonella typhi*. The ethanolic extract leaves and roots moderately to kill all the bacteria (Mangalanayaki and Nirosha, 2013 In Jyotsna *et al.*,2016).

Jyotsna *et al.*(2016) studied the antibacterial activity of both aqueous and chloroform extract of *Carica papaya* on bacteria pathogens. The aqueous extract showed more zones of inhibition on *S. aureus*, *Pseudomonas aeruginosa*, *E. coli* and *S. typhi* than the chloroform extract. In aqueous extract 100 % mg/ml concentration showed 9.8 mm zone of inhibition whereas 100 % chloroform showed 8.4 mm. Sherwani *et al.*, 2013 discovered the antifungal activity of *Carica papaya* against majority of clinical and plant pathogenic fungi. Their work further showed that their activity was better in crushed nature of extract than boiled one. Among 6 saprophytic fungi they tested, fungi activity was observed in only 4 saprophytic fungi namely ;*Penicillium sp.*, *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus* in the range of 15-20mm zone of inhibitions . In case of 5 dermatophytic fungi, activity was observed in only 2 fungi namely; *Microsporium canis* and *Trichophyton mentagrophytes* in the range of 16 -20 mm. While ; in yeast results, among 6 yeasts tested, activity was observed in all *Candida* species including *Candida albicans* ,*Candida albicans* ATCC 0383,*Candida galbrata*, *Candida tropicalis*, *Candida kruzei* in the range of 12 -20 mm (Sherwani *et al.*, 2013)

The present work showed that flavonoid, saponin and protein were absent in ethanol leaf extract of *Citrus aurantifolia*, Pathan *et al.*, 2012 discovered the presence of carbohydrate, alkaloid, flavonoids, steroids, tannins in hydroalcoholic extract of *Citrus aurantifolia* leaves. They further identified that *Citrus aurantifolia* possess good antibacterial and antifungal activities and further confirm that their good bioactive compound are useful in primary health care

Table 1: Zone of inhibition diameter of leaves extracts of *Carica papaya* and *Citrus aurantifolia* on the selected fungi isolates

Fungi species	5 % ethanol		75 mg/ml		150 mg/ml		300 mg/ml	
	A	B	A	B	A	B	A	B
<i>Aspergillus sp.</i>	-	-	1cm	0.8cm	2.2cm	1.5cm	3.1cm	2.5cm
<i>Candida sp.</i>	-	-	1cm	0.9cm	2cm,	1.8cm	3.5cm	2.8cm
<i>Penicillin sp.</i>	-	-	1.2cm	0.8cm	1.5cm	1.1cm	3.2cm	2.9cm

A- Lime leaf extracts. B- Pawpaw leaf extracts. _ no inhibition

Table 2: Qualitative Phytochemical Analysis of *Carica Papaya* and *Citrus Aurantifolia* Leaves in Ethanol Extract

Tests	<i>Carica papaya</i>	<i>Citrus aurantifolia</i>
Flavonoids	+	-
Alkanoids	+	+
Tannins	+	+
Saponin	+	-
Protein	+	-

+ = present
- = absent

Conclusion

Carica papaya and *Citrus aurantifolia* leaves demonstrated antifungal activities against fungi (*Aspergillus*; *Candida* and *Penicillium*), this is an indication that the plants are potential sources for production of drugs for treatment of fungi diseases. The results of the study also supports the traditional application of these plants, however further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antifungal from these plants are the future challenges.

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