International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 5 Issue 5, May 2018

ISSN: 2394-4404

Comparative Study On The Inhibitory Potential Of *Carica Papaya* And *Moringa Oleifera* On *Aspergillus Flavus* And *Aspergillus Niger*

Olubode T. P

Bada T. V.

Adekola F. F.

Bamigboye O.

Department of Science Laboratory Technology, Oyo State College of Agriculture and Technology, Igboora, Nigeria

Arowolo F.

Deparment of Microbiology, University of Ibadan, Nigeria

Abstract: ***Examination of plants across the globe to investigate for a novel bioactive compound that could be a good therapeutic agent to combat infectious diseases especially against multi-drugs resistant pathogens is on rampage. However, the advancement in chemotherapy lies in the search for new drugs to overcome the challenges imposed by multi-drug resistant microbes. This led to the investigation of the inhibitory activity of Carica papaya and Moringa oleifera against clinical fungal isolates (flavus and Aspergillus niger) in other to explore the most potent anti-fungi of the two plants with regards to the two pathogenic fungi. The inhibitory potential of Carica papaya and Moringa oleifera on Aspergillus flavus and Aspergillus niger were determined and compared. The diameter of zone of inhibition of ethanol leaf extract of C. papaya against A. flavus and A. niger ranged from 20.0 mm and 19.5 mm to 21.5 mm and 20.5 mm at concentration of 75 mg/l and 100 mg/l respectively. That of M. Oleifera against both fungi ranged from 12.0 mm and 9.5 mm to 11.0 mm and 16.5 mm at the same concentration. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of C. papaya leaf extract on A. flavus and A. niger, ranged from 25 mg/l to 50 mg/l and 50 mg/l to 75 mg/l. While that of M. oleifera ranged from 75 mg/l to 100 mg/l and 50n mg/l to 75 mg/l respectively. Statistical analysis with one way ANOVA revealed that no significant differences was observed in the inhibitory activity of C. papaya against A. flavus and A. niger at probability P<0.05. However, M. oleifera extract, significantly inhibited the growth of A. niger. This study showed that Carica papaya could be used in herbal medicine to treat infections caused by A. flavus and A. niger since both organisms are susceptible to these plant. In contrast, M. oleifera could be used to treat infection due to A. niger being more susceptible than A. flavus. More research is required to isolate, identify and quantify the bioactive compounds of these plants as well as needs to determine the toxicity before their recommendation for herbal medicine approval.***

Keywords: Carica papaya, Moringa oleifera, antifungal, ethanol leaf extract, clinical isolates

I. INTRODUCTION

Over years, plants have been used as important sources of natural products for maintaining animals and human health. Reportedly, plants contain varieties of bioactive compounds with preventative and curative therapies (Nascemento *et al.*, 2000). However, Medicinal plants being cheap and renewable sources of pharmacologically active substances are known to produce certain chemicals that are toxic to bacteria and fungi (Ahn, 2017). Interest in plants as an antimicrobial could be due to resistance developed by pathogens to commercial antibiotics and the after or physiological effect caused by these drugs to patients (Aliero and Afolayan, 2006).

Carica papaya is an herbaceous succulent plant that belongs to the family of Caricaceae and it possesses self-supporting stems (Sharmin *et al.*, 2015). It is a large perennial

herb with fast growing rate and usually un-branched shrub (erect) of 7-8 m tall within copious latex trunk of about 201m in diameter. The plant has a low life span but can produce fruits for more than 20 years (Bruce and Peter, 2008). In the tropical plant database, documentation on some health beneficial properties and action of *Carica papaya* includes the following: analgesic, amebicide, antibiotic, antibacteria, cardiotonic, cholagogue, digestive ammengogue, vermifuge (Raintree, 2010).

Moringa oleifera on the order hand, is one of the best-known widely distributed and grown species of a monogeneric family; Moringaceae (Anwar *et al.*, 2007). The plant is now widely cultivated in some African countries and South American. It is highly valued because almost every part of the tree has high nutritional value (i.e. leaves, roots, bark, fruits, flower, immature pods and seeds) and is used as food (Anwar *et al*., 2007; Chuang *et al*., 2007). In addition, the plant has been reported to possess antimicrobial properties and this explains the reasons for its wide use in the treatment of human disease classes (Lockett *et al.*, 2000; Anwar *et al.*, 2007).

Aspergillus flavus and *Aspergillus niger* are fungi of human and veterinary importance being common contaminants of food and feed stuffs. They are mostly saprophytic and are found in a variety of habitats commonly as ubiquitous agents of decay (Aliero and Afolayan, 2006).

They (*Aspergillus flavus* and *Aspergillus niger*) occur naturally in soil, decaying vegetation, hay and grains undergoing microbiological deterioration and when conditions are favourable for their growth, invade all types of organic substances where *A. flavus* produce mycotoxins of public health importance. Favourable condition includes high moisture content (at least 7%) and high temperature (Enlirch, 2007). They are human and livestock pathogens associated with Aspergillosis of the lungs and sometimes causing corneal otomycosis and naso-orbital infections (Samson *et al.*, 2001; Klich, 2007). About the documented report on the antimicrobial activity of *Carica papaya* and *Moringa oliefera* leaves ethanolic-extracts; this study is designed to ascertain the most potent inhibitor of *Aspergillus flavus* and *Aspergillus niger* as an antimicrobial agent.

II. MATERIALS AND METHODS

COLLECTION OF PLANT MATERIALS

Fresh leaves of the *Carica papaya* and *Moringa oleifera* were collected from Igbo ora surrounding in Ibarapa Local Government of Oyo State Nigeria and were identified at the Herbarium unit of Botany department, University of Ibadan. The fresh leaves were properly washed under tap water and were oven dried at 400^{O}C for 3days, it was ground into fine powder using electrical blender in the laboratory as described by Mukhtar and Tukar (1999) and were stored at 37^{O}C in a sterile polythene bag until use.

PREPARATION OF THE PLANT LEAVES ETHANOLIC-EXTRACTS

Fifty grams of the finely ground powdered plants of *Carica papaya* and *Moringa oleifera* respectively were weighed separately, in to 200ml absolute ethanol in a volumetric flask and were kept on hot plate with magnetic stirrer for 24hours with regular shaking. After 24hours, each content was filtered with Whatman No. 1 (110 mm) filter paper and the filtrate was evaporated at 45^{O}C in water bath to dryness (Adetunji *et al.*, 2010).

PHYTOCHEMICAL SCREENING OF *MORINGA OLEIFERA* AND *CARICA PAPAYA*

The portion of the dry extract was subjected to phytochemical screening using various methods of Malliga *et al.* (2014). Phytochemical screening was done qualitatively, to test for the presence of alkanoids, flavonoids, saponin, tannin, glycoside, terpenoids and steroids.

TEST ORGANISM

Clinical fungal isolates (*Aspergillus niger* and *Aspergillus flavus*) were obtained from department of medical microbiology laboratory of University of Ibadan college of medicine, Ibadan, Nigeria. The fungal isolates were checked for purity and were maintained on Potato dextrose agar slant in the refrigerator prior to use.

STANDARDIZATION OF INOCULUMS

A little spore of the isolates were picked with sterile inoculating needle and was emulsified with 3-5 ml of sterile physiological saline followed by proper shaking. The turbidity of the suspension was matched and compared with that of 0.5 McFarland standard for sensitivity test as described by NCCLS (2004). The McFarland standard was prepared by mixing 0.6 ml of 1% (w/v) dihydrate barium chloride solution with 99.4 ml of 1% (v/v) sulphuric acid.

BROTH ASSAY OF THE INHIBITORY ACTIVITY OF *MORINGA OLEIFERA* AND *CARICA PAPAYA* ETHANOLIC EXTRACT ON *ASPERGILLUS FLAVUS* AND *ASPERGILLUS NIGER*

Crude extract of *Moringa oleifera* and *Carica papaya* leaves were incorporated into Potato Dextrose Broth at 100mg/l, 75mg/l, 50mg/l and 25mg/l concentrations in conical flasks. The flasks were then inoculated with mycelial discs of the test fungi as four 5mm discs per flask of *Aspergillus niger* and *flavus*. Flask was incubated at 28 ± 2^{O}C for 7days. Mycelia were harvested and the growth of the test isolates were estimated using mycelial dry weight method (Arowora and Adetunji, 2013).

DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION (MIC)

The Minimum Inhibitory Concentration (MIC) of each extract was determined by pipetting 1ml each, of one folds serial dilution of different concentration of the *Moringa oleifera* extract and *Carica papaya* extract, and was dispensed into 9 ml of sterile Potato dextrose broth cooled to 45^{0}C. Five millimetres each of the test fungus was inoculated into the

broth respectively and were incubated at 25^{0}C for 5 days. The lowest concentration of each extract that inhibited the growth of the test organisms was recorded as Minimum Inhibitory Concentration (MIC). Standard/ Commercial antibiotic disc was used as a positive control and the organisms were found to be sensitive to some while Dimethyl Sulfur Oxide (DMSO) was used as a negative control and did not show any zone of inhibition (Linne and Ringstruds, 2007).

DETERMINATION OF THE MINIMUM FUNGICIDAL CONCENTRATION (MFC)

The minimum fungicidal concentration was determined by pipetting 1 ml each, of one-fold serial dilution of each extracts concentration (*Moringa oleifera* and *Carica papaya*) into molten Potato dextrose agar and were poured on plates. The test fungi (5 mm) were inoculated on the agar and plates were incubated at 25^{0}C for 5 days (Carolina *et al*., 2009).

III. RESULT AND DISCUSSION

The antifungal activity of ethanol leaves extract of *Carica papaya* and that of *Moringa oleifera* were tested on *Aspergillus flavus* and *Aspergillus niger*. The result of the antifungal activity of *C. papaya* ethanol leaf extract against the growth of *A. flavus* and *A. niger* are given in table 1 where the diameter of zone of inhibition ranged from 20.0 mm and 19.5 mm to 21.5 mm and 20.5 mm at concentration of 75 mg/l and 100 mg/l respectively. Leave extract of *C. papaya* showed maximum inhibition against *A. flavus* and *A. niger* this disagrees with the work of Baskaran *et al.* (2012) where ethanol leaf extract of *C. papaya* were observed to demonstrate a more potent inhibition against *A. niger* than *A. flavus*. However, this work is similar to the findings of Musfirah *et al.* (2018) where ethanol leaf extract of *C. papaya* inhibited the mycelial growth of *A. flavus* and *A. niger*. The inhibition of *A. flavus* and *A. niger* by leaf extact of *C. papaya* was directly proportional to its concentration. Musfirah *et al*. (2018) reported similar findings and observed that inhibition of *A. flavus* and *A. niger* increased with increase in concentration of the leaf extract.

The data provided in table 2 shows antifungal activity of *Moringa oleifera* against the growth of *Aspergillus flavus* and *Aspergillus niger* in which the diameter of zone of inhibition ranged from 12.0 mm and 9.5 mm to 11.0 mm and 16.5 mm at concentration of 75 mg/l and 100 mg/l respectively. *A. niger* was observed to be more susceptible to *Moringa oleifera* leaf extract than *A. flavus* this disagrees with the research carried out by Isitua *et al.* (2016) where *A. niger* was less susceptible to *M. oleifera* leaf extract than *A. flavus*. However, *A. niger* was inhibited at the highest concentration of the leaf extract. This corroborates with the findings of Isitua *et al*. (2016) who reported that *A. niger* mycelia was inhibited at a high concentration of the extract.

Between the two plants extract, *Carica papaya* extract gave the strongest antifungal activity against *A. flavus* and *A. niger* both of which are causative agents of Apergillosis in human which is deadly particularly to immune suppressed individual. This study showed that *C. papaya* might be a good therapy against infection due to *A. flavus* and *A. niger* being compared with commercial antibiotic nystatin and observed to have related spectrum of potency (table 1), since no significant difference was observed in the antifungal efficacy of *C. papaya* on the two clinical fungal isolates (*A. niger* and *A. flavus*). This disagrees with the research carried out by okunola *et al*. (2012) where no inhibition zone was observed for antimicrobial properties of fresh and dried leaf extract of *C. papaya* on both clinical fungi.

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of ethanol extract of *M. oleifera* and *C. papaya* are shown in table 4. The least concentration at which *A. flavus* growth was inhibited by *C. papaya* leaf extract is 25 mg/l while that of *M. oleifera* is 75 mg/l. Similarly, the least concentration at which *A. flavus* cells were dead is 50 mg/l while that of *M. oleifera* is 100 mg/l an indication that *A. flavus* were more susceptible to *C. papaya* leaf extract than *Moringa oleifera* extract. This might be due to variation in quantity of their phytochemical constituents. Also for *M. oleifera*, *A. flavus* had the least growth inhibitory concentration of 75 mg/l while that of *A. niger* was 25 mg/l an indication that *M. Oleifera* has high efficacy over *A. niger* than *A. flavus*. According to Effiong *et al*. (2016), high MIC is an indication of low efficacy of any plant extract on pathogens and this may result into development of such pathogen to plants extract bioactive compounds.

	Concentration (mg/l)	***A. niger***	**Zones of inhibition (mm) *A. flavus***
	100	20.5±0.01^a	21.5±0.02^a
	75	19.5±0.02^a	20.0±0.00^a
	50	15.5±0.03^b	12.5±0.01^b
	25	12.5±0.02^b	-
Nystatin	50	21.0±0.00^a	22.0±0.01^a

[1]*Values are means±standard deviation of Carica papaya on Aspergillus niger and A. flavus*

[a-b] *Means within a column with different superscripts are significantly different (p<0.05*

Table 1: Antifungal activities of leaf ethanol extract of Carica papaya on Aspergillus niger and Aspergillus flavus

	Concentration (mg/l)	***A. niger***	**Zones of Inhibition (mm) *A. flavus***
	100	16.5±0.01^d	11.0±0.02^a
	75	9.5±0.02^a	12.0±0.01^b
	50	3.8±0.01^c	7.5 ±0.02^a
	25	-	-
Nystatin	50	21.0±0.00^d	22.0±0.02^e

[1]*Values are means±standard deviation of Moringa oleifera on Aspergillus niger and A. flavus*

[a-e] *Means within a column with different superscripts are significantly different (p<0.05*

Table 2: Antifungal activities of leaf ethanol extract of Moringa oleifera on Aspergillus niger and Aspergillus flavus

Plant extracts	***Aspergillus flavus***		***Aspergillus flavus***	
	MIC	**MFC**	**MIC**	**MFC**
	(mg/l)		**(mg/l)**	
Carica papaya	25	50	50	75

Moringa olefera	75	100	25	50

Table 3: Minimum inhibitory and minimum fungicidal concentration of ethanol leaf extract of Carica papaya and Moringa oleifera on A. flavus and A. niger

The qualitative phytochemical analysis of *C. papaya* and *M. oleifera* leaves revealed that they contain flavonoids, tannin, alkaloids, saponin, steroids, terpenoids and carbohydrates. However, *M. oleifera* lacks glycosides. These findings corroborate with the past research carried out where glycosides were not detected in *M. oleifera* leaf. Flavonoids are very important components of natural products and have got both antioxidants activity and ability to get rid of tumor growth (Sikandar *et al.*, 2013). Tannin on the other have been reported to inhibit the formation of fungal cell wall leading to the death of the organisms (Sikandar *et al.*, 2013).

IV. CONCLUSION

This study has shown the difference in the inhibitory potential of *Carica papaya* and *Moringa oleifera* against clinical fungi isolates (*Aspergillus flavus* and *Aspergillus niger*). Hence, *C. papaya* has demonstrated a more potent herbal antifungal property particularly against *A. flavus*. Thus, C. papaya could be a potent herbal medicine against any infection due to *A. flavus*. *C. papaya* could also be used to treat an infection due to A. niger as an alternative herbal medicine in place of *M. oleifera*. The research has shown that herbal medicine can be effective as modern medicine to combat infections due to the two fungi. However, there is need to further isolate, identify and quantify the bioactive compounds of these plants as well as the bioactive principles for development of new pharmaceutical antifungal drugs. In addition, the toxicity of the plants extract must also be determined.

Test	***Carica papaya***	***Moringa oleifera***
Flavonoids	+	+
Alkaloids	+	+
Tannin	+	+
Saponin	+	+
Glycosides	+	-
Steroids	+	+
Terpenoid	+	+
Carbohydrate	+	+

Table 4: Qualitative phytochemical screening of Carica papaya and Moringa oleifera leaf extract

REFERENCES

[1] Adetunji, V. O. and Salawu, O. T. (2010). Efficacy of ethanolic leaf extracts of Carica papaya and Terminalia catappa as molluscicides against the snail intermediate hosts of schistosomiasis. J. Med. Plants Res., 4: 2348-2352.

[2] Ahn, K., (2017). "The worldwide trend of using botanical drugs and strategies for developing global drugs". BMB Reports. 50 (3): 111–116.

[3] Aliero, A. A. and Afolayan, A. J. (2006). Extracts from shoot of Acortisarctotides inhibit the growth of bacteria and fungi. Journal of Pharmacology and Biology 41: 22-25. Anwar, F., Latif, S., Ashraf, M. and Gilan, A. H. (2007). Moringa oleifera: A food plant with multiple medicinal uses. Phytother. Res. 21: 17-25.

[4] Arowora, K. A. and Adetunji, C. O. (2013). Antifungal Effects of Crude Extracts of Moringa oleifera on Aspergillus niger v. Tieghem Associated with Post Harvest Rot of Onion Bulb. Sikkim Manipal University Medical Journal 1 (2): 214-223.

[5] Baskaran, C., Ratha-bai, V., Velu, S. and Kamaran, K. (2012). The efficacy of Carica papaya leaf extract on some bacterial and fungal strain by well diffusion method. Asian Pacific J. Tropical Dis. S658-S662.

[6] Carolina, P. S., Josep, M., Torres-Rodrı´guez, E., Alvarado-Ramı´rez, Francisca, M. G., Maribel, D., Mercedes, P. and Vera, R. (2009). MICs and minimum fungicidal concentrations of amphotericin B, itraconazole, posaconazole and terbinafine in Sporothrixschenckii. Journal of Medical Microbiology 58: 1607-1610.

[7] Chuang, P., Lee, C., Chou, J., Murungan, M., Shieh, B. and Chen, H. (2007). Antifungal activity of crude extracts and essential oil of Moringa oleifera Lam. Journal of Bioresource Technology 98: 232-236.

[8] Effiong, E. B., Hallima, B. M., Buhari, B. Y., Okeke, I., Umeh, S. O., Abubakar, M. and Mbanusi, B. (2016). Comparative phytochemical screening and in vitro antimicrobial activity of stem bark and leaves of Horse Rdish (Moringa oleifera) plant. Academic J. of life 2 (9): 2415-5217.

[9] Ehrlich, K. C. (2007). Aflatoxin-producing Aspergillus species from Thailand. International Journal Food Microbiology 144 (2): 153-159.

[10] Isitua, C. C., Ibeh, I. N. and Olayinka, J. N. (2016). In vitro Antifungal Activity of Moringa oleifera Lam Leaf on some selected clinical fungal strains. Indian J. of Applied research 6 (8):548-552.

[11] Linne, J. J. and Ringstrud, K. M. 2007. Clinical Laboratory Science.5thed.Mosby Elsevier Publisher,St. Louis, Missouri, USA. pp. 486-488.

[12] Lockett, C. T., Calvet, C. C. and Grivett, L. E. (2000). Energy and micronutrient composition of dietary and Medicinal wild plants consumed during drought study of rural Fulani, Northeastern Nigerian International Journal of food Science and Nutrition. 51 (3): 195-208.

[13] Malliga, E., Dhanarajan, M. S., Rajalakshmi, A., Jayachitra, A., Padhasaradhi, M. and Narasimharao, B. (2014). Analysis of phytochemicals, Antibacterial and Antioxidant activities of Moringa oleifera Lam. Leaf extract-an in vitro study. Int_. J. of drug development and research 1: 1-10.

[14] Mukhtar, M. D. and Tukur, A. (1999). In-vitro screening for activity of Pistia stratiotes extract. NISEB Journal 1 (1): 51-60.

[15] Musfirah, A., Qumqum, N., Nafeesa, Z. and Muhammad, R. M. (2018). Investigations of phytochemical and antifungal activity of Carica papaya L. Leaves. J. pure and Applied biology 7 (1): 309-314.

[16] Nascimento, G. G., Locatelli, J., Freitas, P. C. and Silva. G. L. (2000). Antibacterial activity of plant extract and phytochemical on antibacterial-resistant bacteria. Brazil Journal of Microbiology 31 (4): 247-256.
[17] NCCLS-National committee for clinical Laboratory Standards. (2004). Performance Standards for Antimicrobial Susceptibility Testing. (14th Edn.). Wayne, PA, USA. 100-114.
[18] Okunola A. A., Muyideen T. H., Chimedu P. A., Tomisin J. and Harrison,A.(2012). Comparative study on antimicrobial properties of extract of fresh and dried leaves of Carica papaya on clinical bacteria and fungi isolates. Journal of Advanced Science Research 3(5): 3107 -3114.
[19] Raintree Nutriton, (2010). Carica papaya. Tropical Plant Database. http//Olf.www.rain-tree.com/papaya.htm.
[20] Samson, R. A., Houbraken, J., Summerbell, R. G., Flannigan, B. and Muller, J. D. (2001). Common and important species of fungi and actinomycetes in indoor environments. New York:Taylor and Francis. 287-292.
[21] Sharmin, M. R., Islam, M. N. and Alim, M. A., (2015). Shelf-life enhancement of papaya with aloe vera gel coating at ambient temperature. Journal of Bangladesh Agriculture University 13(1): 131-136.