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Fungal Pathogens Associated with Watermelon (*Citrullus lanatus*) Fruit and Efficacy Determination of *Annona senegalensis* Leaf Extract Against The Fungal Isolates

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ABSTRACT

This study focused on the Isolation of fruit-rot fungal pathogens of watermelon (*Citrullus lanatus*) and the efficacy determination of *Annona senegalensis* leaf extract against the fungal isolates. A total of ten (10), each of the spoilt and healthy watermelon fruit from among the samples collected from the four different markets within Mubi were randomly selected for the isolation of fruit-rot fungal pathogens. The collected *A. senegalensis* fresh leaves were air-dried, pulverized and extracted using maceration method of extraction. The extract was then screened for the presence of phytochemicals and antifungal activity test. The study indicated the presence of only two fungal species such as *Aspergillus fumigatus* and *Aspergillus niger* associated with the fruit-rot of watermelon. Terpenoids, flavonoids and tannins were the phytochemicals discovered present in the ethanolic leaf extract of *A. senegalensis*. The antifungal activity test of the extract showed an inhibitory effect against all the fungal isolates with statistically highest diameter zone of inhibition of 17.50 mm and 20.85 mm and lowest zone of 7.80 mm and 3.15 mm on *A. fumigatus* and *A. niger* respectively. Fungal species such as *A. niger* and *A. fumigatus* were responsible for the fruit-rot of watermelon fruit obtained from Mubi region. The ethanolic leaf extract of *A. senegalensis* was effective against *A. fumigatus* and *A. niger*.

INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai], which in Hausa language of Nigeria often known as; "Kankana" is an important hurti-cultural crop, mostly known for its sweet and juicy fruit; and is grown in the warm climates all over the world (Jeffrey, 2001). It is an annual species containing cultivated, semi domesticated and wild forms; and is widely distributed in tropical and sub-tropical areas (Jeffrey, 2001). In Africa, watermelon accounted for about 5.4 % of the harvested area devoted to vegetable production in 2008; and this contributed to the world watermelon production with about 4.6 % of 99,194,223 tonnes (FAOSTAT, 2008). History has it that, watermelon originated from Africa. Although, the exact geographical origin and domestication process of the crop watermelon is not explicit, however, it has been suggested that the origin is in the Sahel Region in Northern Africa (Vander Vossen *et al.,* 2004).

In Nigeria, watermelon is mostly produced in the northern States with Borno State as the major producer of it (Akashi *et al*., 2001 and Adekunle *et al*., 2007). In Borno State: Kaga, Konduga, Marte, Monguno, Kukawa, Mafa, Dikwa, Ngala and Magumeri were the main Local Government Areas where watermelon were mostly cultivated from where they were distributed to different parts of the state and country (Akashi *et al*., 2001).

Watermelon fruit contained about 93 % water, 6 % sugar and small amounts of proteins, fats, minerals, and vitamins (Dudareva *et al.,* 2004 and Namdari *et al*., 2011). Just like most fruits, watermelon fruit supplies some nutritional substances such as minerals and vitamins required in daily human diet for a healthy growth and development (Ewekeye *et al*., 2013). It is believed to be helpful in the control of blood pressure and probably stroke as it contains potassium (Adekunle *et al.,* 2007). Many studies have suggested that increasing consumption of plant foods like watermelon decrease the risk of obesity and overall mortality, diabetes and heart diseases (Afsah-Hejri *et al.,* 2013).

The main problem faced by watermelon farmers in Nigeria, especially at post harvest is that of fruit-rot. Findings had revealed that fungi were the major pathogens responsible for the spoilage of watermelon fruit (Bankole, 1993 and Bankole *et al.,* 2005). Some of these fungi produce aflatoxins (mycotoxins), which are known to be associated with elevated rate of liver cancer, stunted growth and immune-toxicity in West Africa (Turner *et al.,* 2003). Fungal species of the genus *Aspergillus* were reported to cause a significant morbidity and mortality in animals (Person *et al*., 2010). Assessing fruits that are often consumed in raw form for the presence of pathogenic microorganisms is of great importance; as this would aid in knowing how safe the fruits are for human consumption and measures to take in curtailing contaminations. Very little or no literature exists on the fungal species associated with the fruit-rot of watermelon sold in Mubi town of Mubi North and South Local Government Areas of Adamawa State, Nigeria. Therefore, this study was intended in providing information in regard to that.

Annona *senegalensis*, commonly known as African custard apple, is a wild shrub which was reported to contain phytochemicals such as sterols, triterpenes, anthraquinones, flavonoids and alkaloids (Awa *et al.,* 2012). All parts of the plant especially the leaves have been reported to be used in treating yellow fever, tuberculosis and small pox (Aiyeloja and Bello, 2006 and Mustapha *et al.,* 2013). Therefore, considering the fact that most antibiotics are associated with side effects (Cunha, 2001) and sometimes not effective against some of these pathogens; it is imperative that plant based compounds of medicinal importance should be used in the control of these pathogens as they have less side effects, better patient tolerance and are cheaper (Vermani and Garg, 2002). In view of the above, it is not out of place to test the efficacy of the leaf extract of *A. senegalensis* on the pathogenic fungal isolates of watermelon fruit.

MATERIALS AND METHODS

Study Area

The study was conducted in Mubi town which comprised of both Mubi-North and Mubi-South Local Government Areas (L.G.As) of Adamawa State, Nigeria. The town is located in the North Eastern region of Nigeria between latitude $10^{0}14^{1}$N and $10^{0}18^{1}$N of the equator and longitude $13^{0}14^{1}$E and $13^{0}19^{1}$E. It occupies a land of about 725.85 Km^{2}. The area has a tropical climate with an average temperature of 32^{0}C and lies within the Sudan savannah vegetation zone of Nigeria. The area has an average relative humidity ranging from 28 % - 45 % and an annual rainfall of about 1056 mm (Adebayo, 2004).

Sample Collection, Identification and Authentication

A total of twenty (20) samples of spoilt watermelon fruit and twenty (20) healthy ones were collected from four (4) different markets within Mubi [i.e five (5), each of the spoilt and healthy ones from each of the markets]. These markets include; Kasuwan Kuturu, Kasuwan Dawa, Mubi main market and Kasuwan Tikke respectively. The leaf samples of the *A. senegalensis* were obtained from Gombi local government area of Adamawa State. The collection was made into sterilized polythene bag. It was then taken to the herbarium unit of the department of Botany, Adamawa State University, Mubi for authentication.

Preparation of Plant Samples for Fungal Isolation and Extraction

The collected and identified fresh leaf samples of the *A. senegalensis* were washed using à running tap water. After proper washing, they were then shade-dried at room temperature. The dried leaf sample was then pulverized (grinded) into fine powder using the wooden type of pestle and mortar and stored in a sterilized polythene bag pending its usage.

In preparation of the isolation of fungi from the watermelon fruit, a total of 10 spoilt watermelon fruit and another 10 healthy looking ones were randomly selected from among the sample collected from the four markets within Mubi. These were used to determine the presence of fungi on the fruit. Therefore, the watermelon fruit were then cut into small segments (of about 3 mm in diameter) with à sterilized blade and surface sterilized in 1% hypochlorite for 2 minutes (Al-Hindi *et al.,* 2011).

Extraction of the Plant Material

Maceration method of extraction was used for the extraction of the plant constituents using ethanol as a solvent. About 300 g of the pulverized (grinded) *A. senegalensis* leaf powder was weighed and place in one liter of ethanol contained in a conical flask and covered with aluminum foil. The mixtures were shaken vigorously from time to time and allowed to stay for a period of 24 hours. The mixture after 24 hours was filtered first using mushin cloth and finally using Whatman No. 1 filter paper. The filtrate was then concentrated using rotary evaporator at 60^0C. The concentrate was then collected in a sample bottle and kept in a refrigerator at 4^0C pending analysis.

Isolation of Fungi

The sliced watermelon fruit prepared above was placed on Sabouraud Dextrose Agar (SDA) aseptically and then incubated at 28°C for 5 days. A pure culture was then obtained and maintained by sub-culturing each of the different colonies that emerged on the SDA plates and incubating at 28°C for 5 days. As a control, the healthy fruits were sterilized with 75 % ethanol. The fruit was cut into small segments with a sterilized blade and placed on SDA and then incubated at 28°C for 5 days.

Pathogenicity Test

A Fresh watermelon fruit was rinsed with distilled water and sterilized with 70 % ethanol. With the aid of sterile cork borer, 4 mm diameter cylindrical holes were dug into the healthy watermelon fruits and the plugs were pulled out. About 4 mm diameter mycelia disc of the pure fungal isolates was introduced into the hole dug on the watermelon fruit by placing it at the bottom of the hole. The plugs were then carefully replaced and the wounded area sealed up with wax to prevent contamination by other organisms. The inoculated fruits were incubated at room temperature (28^0C) for 5 days. The inoculated watermelon fruits were eventually observed for rot development.

Identification of Isolated Fungi

The fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology and pigmentation as described by Tafinta *et al.* (2013).

Phytochemical Screening

The *A. senegalensis* ethanolic leaf extract was screened for the presence of the compounds such as terpenoids, steroids, tannins, flavonoids and anthraquinones using the methods adopted by Khandelwal (2003).

Antifungal Activity Testing

Preparation of different concentrations of the leaf extract:

The ethanolic leaf extract used for the antifungal activity test was prepared into four (4) different concentrations ranging from 25 to 200mg/ml (i.e 25, 50, 100 and 200 mg/ml) in two (2) replications. The extract concentration was prepared by weighing 2 g of the extract into 10 ml of sterile distilled water (200 mg/ml). A doubling dilution of the diluted extract was carried out into three (4) different labeled bottles to obtain concentrations 100, 50 and 25 mg/ml respectively.

Standardization of the inocula:

Standardization of the fungal inoculums was carried out by picking a pinch of the fungal colony from the pure sub-cultured fungal plate and placed into a test tube containing 10 ml of sterile distilled and shaken vigorously so as to obtain a discrete fungal colony.

Susceptibility testing of the extract:

This was carried out using agar well diffusion method and Ketoconazole as control. The standardized fungal organism was uniformly streaked onto freshly prepared SDA with the aid of a sterile swab stick (cotton swabs). For wells were punched on the inoculated SDA plates using a sterile cork borer of 6 mm in diameter. The wells were properly labeled according to the different concentrations of the extract prepared. The punched wells were filled with 0.2 ml of the extract. The plates were then allowed to stay on the bench for about 1hour for the extract to diffuse into the agar after which they were incubated at 30^0C for 24 hours. After the incubation period, the plates were observed for any evidence of inhibition, which appeared as clear zones that was completely devoid of growth around the wells. The diameter of the clear zones were measured with a transparent ruler, calibrated in millimeter (mm).

RESULTS AND DISCUSSION

The isolation of fungi from the rotten watermelon fruits obtained from the four different markets within Mubi town revealed the presence of only two main fungal species, namely: *Aspergillus fumigatus* and *Aspergillus niger* (Plates I and II). The *A. fumigatus* was observed through the microscope to possessed the characteristics such as: gray stipes around the apex, have a smooth surface, a small and columnous globuse and surface of the conidia was smooth. The *A. niger*, however, was observed to possess the following characteristic features: a smooth and colorless conidiosphores, the mycelium body was filamentous and dark brown conidial head containing a dark brown spores. When pathogenicity test was determined using these fungal isolates, it was observed that all the inoculated watermelon fruits got rotten after the few days of incubation at room temperature.

The presence of these two fungal species of *Aspergillus* as the only fungi associated with rotten watermelon fruit from these markets could be attributed to the sufficient sugary flavor of the watermelon fruit which favour their growth (Singh and Sharma, 2007). The temperature of the study area could also be another contributing factor to the presence and spoilage caused by these species of *Aspergillus* as similarly reported by Dudareva *et al.* (2004) who attributed the spoilage of watermelon fruit and other fruits to high temperature of the region which according to them favour fungal growth. They further emphasized that, the higher the temperature, the faster is the spoilage. Isolation of *Aspergillus* species such as *A. niger* and *A. fumigatus* from rotten watermelon fruits was similarly reported by Jidda and Adamu (2017).

The qualitative phytochemical screening of the ethanolic leaf extract of *A. senegalensis* showed the presence of most of the compounds for which the extract was screened for. These compounds include: terpenoids, flavonoids and tannins (Table 1). Their presence in the leaf extract of *A. senegalensis* justified its antifungal effect as terpenoids and especially flavonoids were reported to be active compounds in plants responsible for protection against microbial infection in both plants and animals (Reichard, 2013). The inability of the other compounds to be detected might be attributed to the insignificant quantity of the compound in the extract, the method of extraction or the solvent used for the extraction of the phytochemical constituents. Similar situation was reported by Tizhe *et al*. (2015) and Ndamitso *et al.* (2013). They attributed the absence of some compounds in their plant extracts to the type of solvents and the methods of extraction used. They justified their claims by carrying out quantitative screening of their plant extracts and discovered presence of those compounds not detected by the qualitative screening.

The antifungal activity test of *A. senegalensis* ethanolic leaf extract showed an inhibitory effect on the two test organisms (*A. fumigatus* and *A. niger*) at all the four (4) different concentrations (25 mg/ml to 200 mg/ml) except on *A. fumigatus* at the lowest concentration (25 mg/ml). The statistically highest ($p<0.05$) zones of inhibition observed were 17.50 mm and 20.85 mm on *A. fumigatus* and *A. niger* respectively at the highest concentration (200 mg/ml) and the lowest zones were 7.80 mm and 3.15 mm on *A. fumigatus* and *A. niger* at concentrations 50 mg/ml and 25 mg/ml respectively. The control (ketoconazole) showed a significantly higher zones of inhibition (14.00 mm and 15.50 mm) on *A. fumigatus* and *A. niger* compared to those of concentrations 100 to 25 mg/ml, but lower than that of the highest concentration (200 mg/ml) on the two test organisms (Table 2). The inhibitory effect of this ethanolic leaf extract could be attributed to the presence of those active compounds detected qualitatively in the plant extract. The findings could mean that, the higher the concentration, the higher is the effect of the extract on test organisms.

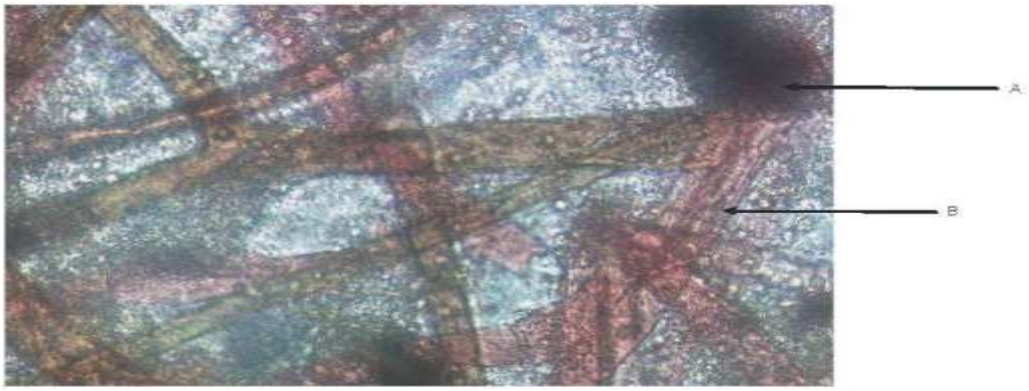

Plate I: Photomicrograph of *A. fumigatus* x40
Key: A= Spores, B= Hyphae

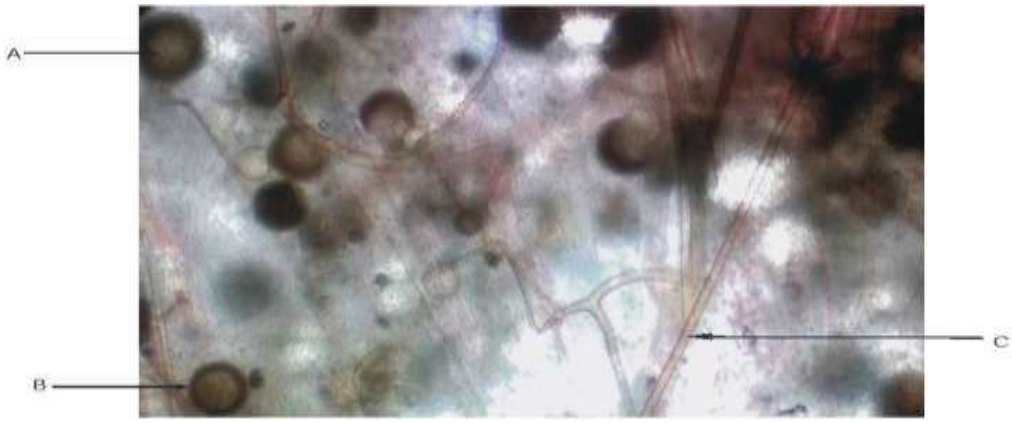

Plate II: Photomicrograph of *A. niger* x40
Key: A = Conidiophore, B = Spores, C = Hyphae

Table 1: The qualitative phytochemical screening of the ethanolic leaf extract of *A. senegalensis*

Phytochemical Constituents	Status
Terpenoids	+
Steroids	-
Anthraquinones	-
Flavonoids	+
Tannins	+

Key: + = Present; - = Not detected

Table 2: The antifungal activity test of the ethanolic leaf extract of *A. senegalensis* on two *Aspergillus* species associated with fruit-rot of watermelon

	Diameter of Zone of Inhibition (mm) Test Organisms	
Extract concentration (mg/ml)	***A. fumigatus***	***A. niger***
200	17.50^{a}	20.85^{a}
100	11.75^{c}	13.00^{c}
50	7.80^{d}	7.05^{d}
25	0.00^{e}	3.15^{e}
Control	14.00^{b}	15.50^{b}
SE±	0.60	0.60

NB: Means with different superscript along the column are statistically significantly different at $p<0.05$. SE± = Standard Error

CONCLUSION

In conclusion, therefore, *Aspergillus* species (*A. niger* and *A. fumigatus*) were responsible for the fruit-rot of watermelon fruit sold in the four markets of Mubi. And the ethanolic leaf extract of *A. senegalensis* were effective against the *Aspergillus* species such as *A. niger* and *A. fumigatus* especially at the higher concentrations.

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Conflict of Interest

The authors declared no conflict of interest.

REFERENCES

Adebayo AA (2004). Mubi Region Geographic Synthesis, first edition paracelet publishers, Yola, Nigeria, p 19.

Adekunle AA, Fatunbi AO, Adisa S, Adeyemi OA (2007). Growing watermelon commercially in Nigeria: An illustrated guide. USAID ICS Nigeria.

Afsah-Hejri L, Jinap S, Hajeb P, Radu S, Shakibazadeh S (2013). A review on mycotoxins in food and feed: Malaysia case study. Compr*ehensive* Review in Food Science and Food Safety, 12(6):629–651.

Aiyeloja A and Bello O (2006). Ethnobotanical potentials of common herbs in Nigeria: A case study of Enugu State, Educational Research Revolution, 1(1):16-22.

Akashi K, Miyake C,Yokota A (2001). *Citrulline,* a novel compatible solute in drought-tolerant wild watermelon leaves, is an efficient hydroxyl radical scavenger. FEBS Letter, 508: 438-442.

Al-Hindi RR, Al-Najada AR, Mohamed SA (2011). Isolation and identification of some fruit spoilage fungi:Screening of plant cell wall degrading enzymes. African Journal of Microbiology Research, 5(4):443–448.

Awa EP, Ibrahim S and Ameh DA (2012). GC/MS analysis and antimicrobial activity of diethyl ether fraction of methanolic extract from the stem bark of *Annona senegalensis.* International Journal of Physical Science Research, 3(11):4213-4218.

Bankole SA, Osho A, Joda AO, Enikuomehim OA (2005). Effect of drying method on the quality and storability of "Egusi" melon seeds (*Colocynthis citrusllus* L.). African Journal of Biotechnology, 4(8):799-803.

Bankole SA (1993). Moisture content, mould invasion and seed germinability of stored melon, Mycopathologia, 122: 123-126.

Cunha BA (2001). Antibiotic side effects. Medicinal clinics North America, 85:149-185.

Dudareva N, Pichersky E, Gershenzon J (2004). Biochemistry of plant volatiles. Plant Physiology. 135: 1893-1902.

Ewekeye TS, Oke OA, Quadri AI, Isikalu AO, Umenwaniri MO, Durosinmi MI (2013). Studies on post-harvest deterioration of some fruits and vegetables in selected markets in Lagos State, Nigeria. American Journal Research and Communication, 1:209-222.

FAOSTAT (2008). Crops FAOSTAT. *Food and agriculture organization of the United Nations.*

Jeffrey C (2001). Cucurbitaceae. In Hanelt P (Ed.) Mansfeld's Ornamentals, Vol. 3. Springer, Berlin, Germany, pp 1510-1557.

Jidda MB and Adamu MI (2017). Rot inducing fungi of watermelon (*Citrullus lanatus*) fruits in storage and fruit stalls in Maiduguri, Nigeria. International Journal of Advanced Academic Research – Sciences, Technology and Engineering, 3(5):11-18.

Khandelwal KR (2003). Practical pharmacognosy techniques and experiments, 10th edition. Nirali Prakashan, Pune. Pp.149-158.

Mustapha A, Owuna G and Uthman I (2013). Plant remedies practiced by Keffi people in the management of dermatosis. Journal of Medicinal Plant Studies, 1(5):112-118.

Namdari M, Mohammed A, Mobtaker HG (2011). Assessment of energy requirements and sensitivity analysis of inputs for watermelon production in Iran. International Journal of Plant, Animal and Environmental Science, 1:1

Ndamitso MM, Musah M, Mohammed-Hadi Z, Idris S, Tijjani OJ, Shaba EY and Umar A (2013). Analysis of phytochemical content and antibacterial activity of *Tapinanthus dodoneifolius* extracts. Researcher, 5(5):54-59.

Person AK, Chudgar SM, Norton BL, Tong BC and Stout JE (2010). *Aspergillus niger*: an unusual cause of invasive pulmonary aspergillosis. Journal of Medicinal Microbiology, 59:834-838.

Reichard Z (2013). Terpenes: What are terpenoids and what do these do? MedicalJane publication, USA. Pp. 45-53.

Singh D, Sharma RR (2007). Postharvest diseases of fruit and vegetables and their management. In:Prasad, D. (Ed.), sustainable pest management. Daya publishing house, New Delhi, India.

Tafinta IY, Shehu K, Abdulganiyyu H, Rabe AM, Usman A (2013). Isolation and Identification of fungi associated with the spoilage of sweet orange (*Citrus sinensis*) fruits in Sokoto State, Nigeria. Journal of Basic and Applied Science, 21(3):193–196.

Tizhe TD, Alonge SO and Aliyu RE (2015). Antibacterial activity of *Globimetulla braunii* sourced from five different host trees in Samaru, Zaria, Nigeria. International Journal of Current Science, 18:117-123.

Turner PC, Moore SE, Hall AJ, Prentice AM, Wild CP (2003). Modification of immune function through exposure to dietary aflatoxin in Gambian children, Environmental health perspective. Journal of Environmental Health, 111:217-220.

Van der Vossen HAM, Denton OA, El Tahir IM (2004). *Citrullus lanatus* (Thumb.) Matsum and Nakai .In Grubben GJH, Denton OA (Ed.) Plant Resources of Tropical Africa 2. Vegetables PROTA Foundation, Wageningen, pp. 185-191.

Vermani K and Garg S (2002). Herbal medicines for sexual transmitted diseases and AIDs. Journal of Ethnopharmacology, 50:27-34.

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