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RESEARCH ARTICLE

Antifungal Activity of *Cucumis metuliferus* E.Mey. ex Naudin on Some Post-harvest Decay Fungi of String beans

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Abstract

Crude aqueous and ethanolic extracts of *Cucumis metuliferus* fruit were screened for their phytochemical components which revealed the presence of alkaloids, tannins, flavonoids, saponins and cardiac glycosides. Steroids and anthraquinones were completely absent. The extracts were tested for their inhibitory effect on five fungal species (*Aspergillus flavus, Fusarium oxysporum, Mucor* sp., *Penicillium citrinum* and *Rhizopus stolonifer*) isolated from decayed *Phaseolus vulgaris* (String Bean). The different concentrations of the extract used ranged between 200-1000 mg/mL. Screening for antifungal activity using agar well diffusion method showed inhibition of all the test fungi especially at higher concentrations except for *R. stolonifer* that was not inhibited by the ethanolic extract. The highest effect of aqueous extract was observed in *P. citrinum* with inhibition zone of 34 mm at 1000 mg/mL, followed by *Mucor* sp. with inhibition zone of 32 mm, the least was recorded for *F. oxysporum* with zone diameter of 22 mm. The highest activity for ethanolic extract was recorded for *A. niger* with inhibition zone of 35 mm while the least was recorded for *F. oxysporum* with zone diameter of 18 mm. The Minimum Inhibitory Concentration (MIC) of the extracts was determined using agar dilution method. The MIC values of the extracts ranged between 12.5-25 mg/mL. The results showed *A. niger* and *P. citrinum* as the most sensitive and *F. oxysporum* as the least sensitive to the extract. The Minimum Fungicidal Concentration (MFC) of the extracts also ranged between 25-50 mg/mL indicating that the extracts are fungicidal at those concentrations.

Keywords: *Cucumis metuliferus*, antifungal activity, minimum inhibitory concentration, fungicidal.

Introduction

Herb is a plant or any part of a plant valued for its medicinal, aromatic or savory qualities (Bodeker *et al.,* 2005). The use and search for drugs and dietary supplements derived from plants have accelerated in recent years. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties (Cowan, 1999). According to the World Health Organization (WHO, 1998), ''Herbal Preparations'' contain plant parts in the crude or processed state as active ingredients and may also contain additives (excipients). Secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medical agents with lesser side effects and low resistance in microorganisms (Mahesh and Satish, 2008; Seyyednejad and Motamedi, 2010). These active principles which are contained in medicinal plants can be used as an alternative cheap and effective herbal drug against microbial infections. In Ethiopia, traditional remedies represent not only part of the struggle of the people to fulfill their essential drug needs but also they are integral components of the cultural beliefs and attitudes (Tadeg *et al.,* 2005; Demma *et al.,* 2009).

Contamination of food by food-borne pathogens and microorganisms that cause decay is of great concern in the food industry. Fungi, especially *Aspergillus, Fusarium, Mucor, Penicillium* and *Rhizopus* species, are among the major causes of food decay, especially bakery products, high moisture food products such as fruits and vegetables and intermediate moisture products such as cheese, grains, preserved fruits (Legan and Voysey, 1991). Apart from the repelling sight of the fungal visible growth, they also cause off-flavor formation and production of mycotoxins, with carcinogenic and teratogenic attributes and residual toxicity which are of great health concern. *Aspergillus niger* and *Mucor* sp. are worldwide in distribution and have been isolated from various habitats including stored foodstuffs. *Aspergillus* has been reported as one of the predominantly isolated microbial species in Nigeria (Ogaraku, 2010). Sharma and Vir (1986) reported *A. niger* as the cause of rotting in numerous food products including fruits and vegetables, thereby causing huge economic losses due to spoilage. The fungus is opportunistic pathogen in man, causing asthma, pulmonary aspergilloma and otomycosis in compromised individuals (Austwick, 1965; Korzeniowska, 1990).

In recent years, many researchers have put emphasis on the search for natural antimicrobial compounds that can properly serve the needs of food manufacturers and consumers. Herbs have been used in foods since ancient times as food preservatives and have been recognized by their medicinal values, particularly as antimicrobial agents and natural preservatives. They have been used throughout the past as an alternative approach to preserve foods. Several studies have revealed the results on the preservative action of spices or their essential oils. Dabur (2004) reported the inhibitory effect of methanolic extracts of *Solanum xanthocarpun* and *Datura metelon* on the growth of *Aspergillus fumigatus, A. flavus* and *A. niger* and their *in vitro* MICs were found to be 1.25-2.50 mg/mL by microbroth dilution. Effects of aqueous and ethanolic extracts of citrus species against *Mucor* and *Rhizopus* species was also reported (Mohanka and Priyanka, 2014). Other reports include those of Skandamis *et al.,* (2001), Elgayyar *et al.* (2001), Puupponen *et al.* (2001) and Kuete *et al*. (2010).

Cucumis metuliferus, an annual climber belong to Cucurbitaceae (Cucumber Family, Gourd Family). It is an annual vine and grows to a height of about 5-10 ft high. It requires rich, well-drained moisture retentive and very warm soil. This exotic fruit is similar to the Cucumber and Melon family. Generally, horned melon as it is known is also called as African cucumber, jelly melon, hedged gourd, English tomato, Melano and kiwano. African cucumber is native to Africa and Southeastern America. It is widely distributed to the tropical regions of California, Chile, Canada, Australia and New Zealand (Wilkins-Ellert, 2004). Comparatively, this oval melon has a taste similar to banana and the texture is similar to *Cucumis sativus* (cucumber) as well as zucchini. Traditionally and commercially, it is recently produced on a large-scale as the fruit is used for various edible and medicinal purposes. This very interesting plant is found in many parts of Africa. There are two forms, a bitter form and a non-bitter form. The bitter form is not edible and is in most cases poisonous. The non-bitter form however is edible. The cucumber has a number of medicinal properties that are used throughout Africa. It contains the toxin saponin, giving it many medical properties. The Shona and Mutare area (Zimbabwe) use the horned cucumber's root to help with pain after child birth (Roodt, 1998; Wilkins-Ellert, 2004). It has also been argued that the root when boiled is a brilliant cure for gonorrhea. The seeds are vermifuge. Traditional medical practitioners in Zimbabwe consider the bitter wild fruits as poisonous if taken by mouth. In Benin the fruit is said to possess medico-magical properties and is used to treat eruptive fevers in 'Sakpata voodoo' rituals. The decorticated fruit macerated in distilled palm wine or lemon juice is used to treat smallpox and skin rashes. The refreshing jelly is best scooped out of the shell with a spoon or used in fruit salad. The leaves if cooked are primarily used as a snack or salad (Wilkins-Ellert, 2004).

In Nigeria, the antiviral activity of the fruit of *C. metuliferus* has been reported (Wannang *et al.,* 2010; Amagon *et al.,* 2012; Anyanwu *et al.,* 2014; Usman *et al.,* 2014). The antimicrobial effect of the fruit on *Salmonella gallinarum* was reported by Usman *et al.* (2014). Other reports include that of Aliero and Gumi (2012) on the germination, chemical composition and antimicrobial properties on *C. metuliferus* in Sokoto State. The present study was embarked upon to determine the *in vitro* antifungal activity of fruit of *Cucumis metuliferus* on some isolated fungi causing post-harvest deterioration of *Phaseolus vulgaris* (String bean) in Jos, Nigeria in order to explore its use as a natural preservative in food storage.

Materials and methods

Collection and identification plant material: The ripe intact fruits of *C. metuliferus* were bought from fruits and vegetables garden behind Jos Museum, Nigeria and were taken to Dept. of Plant Science and Technology, University of Jos for identification. The plant was identified by Prof. C.O. Akueshi. The specimen voucher no CCM4 was kept in the herbarium of the department for future reference. The fruits were then taken to the laboratory for processing.

Preparation of the fruit pulp: The ripe fruits were washed in three changes of sterile distilled water to reduce the surface contaminants and were air dried. The fleshy mesocarp of ripe fruits of *C. metuliferus* was carefully scooped out with the aid of a clean spatula. The whole content was blended using laboratory electric blender. The homogenate was passed through a sieve of 0.25 mm pore size. The smooth filterate was evenly spread on an aluminium tray and was completely dried in the hot air oven at 60^{o}C until. The resultant product was then pulverized with the aid of mortar and pestle (Anyanwu *et al.,* 2014). The powder which weighed 2.2 kg was stored in air tight bottles until required.

Extraction of the plant material: The method of Usman *et al.* (2014) was adopted for the extraction but with modifications. Aqueous and ethanolic extracts of *C. metuliferus* were prepared by steeping 200 g of the powder in 500 mL sterile distilled water and 70% ethanol in sterile 1000 mL flasks separately. The flasks were kept for 48 h at room temperature with occasional shaking. The plant extracts were filtered through Whatman No. 1 filter paper. The solution was then evaporated using rotary evaporator (model R 110) at 50°C. The aqueous extract yielded 20.15% while ethanolic extract yielded 9.8%. The extracts were kept in the desiccator until required.

Determination of physical properties of the extracts: The color of the two extracts was visually assessed immediately after the removal of the solvent by rotary evaporation process. The pH was tested using Jenway pH meter. Texture was felt manually in between the

fingers, with the help of glass rod that was used to remove a little portion of each of the extracts (Adoum and Fatope, 1997).

Phytochemical screening of the extracts: The aqueous and ethanolic extracts of ground powder were screened for their phytochemical constituents using standard methods of analysis (Sofowora, 2008; Evans, 2009).

Test for alkaloids: About 0.5 g of each extract was stirred with 3 mL of 1% aqueous hydrochloric acid (HCL) on a steam bath and filtered. Of the filtrate, 1 mL was taken individually into 2 test tubes. To the first portion, few drops of Dragendorff's reagent were added; occurrence of orange-red precipitate was taken as positive. To the second 1 mL, Mayer's reagent was added and appearance of buff-colored precipitate will be an indication for the presence of alkaloids

Test for carbohydrates: About 100 mg of each extract was dissolved in 3 mL of distilled water and mixed with a few drops of Molisch reagent (10% solution of α-naphthol in alcohol). Then 1 mL of conc. H_2SO_4 was carefully added down the sides of the inclined test tube so that the acids form a layer beneath the aqueous solution without mixing it. A reddish or violet ring at the interphase of the two layers was observed indicating the presence of carbohydrate (Sofowora, 1993).

Test for tannins: About 0.5 g of each plant extract was stirred with 1 mL of distilled water, filtered and few drops of ferric chloride solution were added to the filterate. A blue-black, green or blue green precipitate was taken as evidence for the presence of tannins

Test for cardiac glycosides: About 100 mg of each extract was taken in a test tube. A volume of 2.5 mL of dilute H_2SO_4 was added and boiled in a water bath for 15 min. This was cooled and neutralized with 20% potassium hydroxide solution. About 5 mL of a mixture of fehling's solution A and B was added and boiled for 3 min. A brick red precipitate indicated the hydrolysis of a reducing sugar, an indication of glycoside.

Test for flavonoids: About 2 g of the powered fruit was completely detanned with acetone. The residue was extracted in warm water after evaporating the acetone on a water bath. The mixture was filtered while hot. The filtrate was cooled and used for the following tests.

Lead acetate test for flavonoids: About 5 mL of the detanned water extracted was introduced into a test tube, a volume of 3 mL lead acetate solution was added. A yellow colored precipitate indicated the presence of flavonoids.

Sodium hydroxide test for flavonoids: About 5 mL of 20% sodium hydroxide was added to equal volume of each of the detanned extracts. A yellow solution was obtained. A change in color from yellow to colorless on addition of dilute hydrochloric acid indicated the presence of flavonoids.

Test for saponins: A weight of 0.5 g of each of the extract was shaken vigorously with 3 mL of distilled water in a test tube. Frothing which persist on warming was taken as preliminary evidence for the presence of saponins.

Test for steroids: About 100 mg of the extract was dissolved in 2 mL of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown color at the interface is indicative of the presence of steroids.

Test for anthraquinones: Borntrager's test was used for the detection of anthraquinones. About 0.5 g of each extracts was taken into a dry test tube and 5 mL of chloroform was added and shaken for 5 min. The extract was filtered and the filterate was shaken with an equal volume of 10% ammonia solution. A pink violet or red color in the ammonical (lower) layer indicates the presence of free anthraquinones.

Antimicrobial studies

Test fungi: The test fungi used in the study included *Aspergillus flavus, Fusarium oxysporum, Mucor* sp., *Penicillium citrinum* and *Rhizopus stolonifer*. These fungal species were isolated from deteriorated *Phaseolus vulgaris* (String beans) in Microbiology Laboratory of Department of Plant Science and Technology, University of Jos, Nigeria. They were identified as the causative agents for the deterioration of the fresh beans. The isolates were maintained under appropriate environmental condition and stored at 4^oC until required.

Culture medium and reagent: Sabouraud Dextrose Agar medium (Oxoid) and Sabouraud Dextrose broth used in this research work were prepared according to the manufacturer's instruction. The culture media (Sabouraud Dextrose Agar medium), Sabouraud Dextrose broth and Tween-80 were obtained from Microbiology Laboratory of Dept. of Plant Science and Technology, University of Jos, Nigeria.

Preparation of concentrations of the extracts and standard drug: Stock solution of each of the extracts was prepared by weighing 0.2, 0.4, 0.6, 0.8 and 1.0 g of each of the extract s (aqueous and ethanolic) using a digital weighing scale and to each of the extracts 1 mL of distilled water was added to obtain the following concentrations respectively 200, 400, 600, 800 and 1000 $mgmL^{-1}$ from each fruit extract. Sterile distilled water served as negative control while ketoconazole (50 mg/mL) served as positive control.

Standardization of inoculum: A loopful of the pure cultures of each of the test fungus was sub-cultured on

separate acidified Potato Dextrose Agar (PDA) plates and the plates were incubated at 30±1°C for 5 days. The agar plates containing fungal cultures were then washed with 10 mL 0.05% Tween-80 to obtain a spore suspension (Bullerman *et al.,* 1977). The OD 625 of the spore suspension was measured using UV spectrophotometer and adjusted to 0.1 by using 0.05% Tween-80 to achieve an inoculum size of 10^5 cfu/mL was confirmed by plating out the suspension on acidified PDA using standard methods (NCCLS, 2004).

Antifungal screening: The antifungal activity of the extracts was determined by modified agar well diffusion method (Barry, 1980; Zamanian, 2005). A volume of 100 µL of inoculum suspension already prepared was seeded uniformly on sterile Sabouraud Dextrose Agar plates and were allowed to dry for 30 min. Wells (6 mm diameter) were made on the seeded agar plates using a sterile standard cork borer. The bottoms of the wells were sealed by pouring 50 µL of molten Sabouraud Dextrose Agar into scooped out wells. A volume of 200 µL of each of the concentrations (200, 400, 600, 800 and 1000 $mgmL^{-1}$) of the aqueous extract was added to the wells. The same set of experiment was done for the ethanolic extract. A plate to one concentration and each experiment was carried out in triplicates. Fluconazole, 1 mg/mL was used as positive control. The plates were incubated at 30±1°C for 5 days. The mean zone diameter (MZD) of inhibitions was taken and recorded. Those extracts that showed maximal zones of inhibition were further subjected for testing of Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC).

Statistical analysis: The data obtained were subjected to analysis of variance (ANOVA) and differences between means of treatments were calculated using least significance difference (LSD) test at 5% level of probability (Aliero and Gumi, 2012).

Determination of Minimum inhibitory concentration (MIC): This test evaluates the lowest concentration of the extract that inhibits the growth of the test organisms as measured by observed turbidity in the test tubes. MIC was determined using the doubling dilution method of NCCLS (2004). A volume of 2 mL of sterile Sabouraud Dextrose broth in test tubes were added, 2 mL of each of the extracts in separate test tubes to get stock concentration of 100 mg/mL. A series of 9 mL Sabouraud Dextrose broth was arranged in a test tube rack with the 1st tube having double strength broth and others with single strength broth. Doubling dilution was done by pipetting 1 mL of the test agent into the test tubes to have extract concentrations of 50, 25, 12.5, 6.25 and 3.125 mg/mL. The tubes were then inoculated with 3 loopfuls of the test organism. The tubes were incubated at 30±1ºC for 5 days. Uninoculated tubes, one without any antimicrobial agent which served as negative control and another with fluconazole (1 mg/mL) which served as positive control were also incubated. MIC was regarded as the lowest concentration at which there was no turbidity or growth of the test fungi after the 5 days incubation period, as compared to the controls.

Determination of Minimum fungicidal concentration (MFC): The test tubes used in the determination of MIC that showed no visible growth or turbidity were used for the determination of MFC. A loopful of the contents of the tubes showing no microbial growth was sub-cultured by streaking over the surface of already poured and set SDA plates without extracts. The plates were labeled appropriately and were incubated at 30±1ºC for 5 days. The MFC was recorded as the lowest concentration at which no growth was seen on the sub-cultured plates.

Results

Physical parameters of the extracts of Cucumis metuliferus fruit: Physical parameters of extracts of *Cucumis metuliferus* fruit indicated that the texture was gum-like and jelly-like and also, the pH was 5.84 and 6.61 for both aqueous and ethanolic extracts respectively (Table 1). The color of the aqueous extract was light brown while that of the ethanolic extract was deep brown. The percentage yield as shown in Table 1 was 20.15% for aqueous extract and 9.8% for ethanolic extract.

Table 1. Physical parameters of the extracts of *C. metuliferus* fruit.

Extract	Texture	Physical Parameters color	pH	Yield
Crude aqueous extract	Gum-like	Light brown	5.84	20.15
Crude ethanolic extract	Jell-like	Deep brown	6.61	9.8

Table 2. Phytochemical constituents of *Cucumis metuliferus* extract.

Constituents	Aqueous	Ethanolic
Alkaloid	+++	++
Tannins	++	++
Flavonoids	+++	++
Carbohydrates	++	+++
Steroids	-	-
Anthraquinones	-	-
Saponins	+	++
Cardiac glycosides	++	++

+=Trace amount, ++=Moderate amount, +++=large amount, - = absent.

Table 3. Minimum inhibitory concentration (MIC) of fruit pulp of *C. metuliferus* against test fungi.

Extract (mg/mL)	*Aspergillus flavus*	*Fusarium oxysporum*	*Mucor* sp.	*Penicillium citrinum*	*Rhizopus stolonifer*
Aqueous	12.5	25	25	12.5	25
Ethanol	12.5	25	25	12.5	25

Table 4. Minimum fungicidal concentration (MFC) of fruit pulp of *C. metuliferus* against test fungi.

Extract (mg/mL)	*Aspergillus flavus*	*Fusarium oxysporum*	*Mucor* sp.	*Penicillium citrinum*	*Rhizopus stolonifer*
Aqueous	25	50	50	25	50
Ethanol	25	50	50	25	50

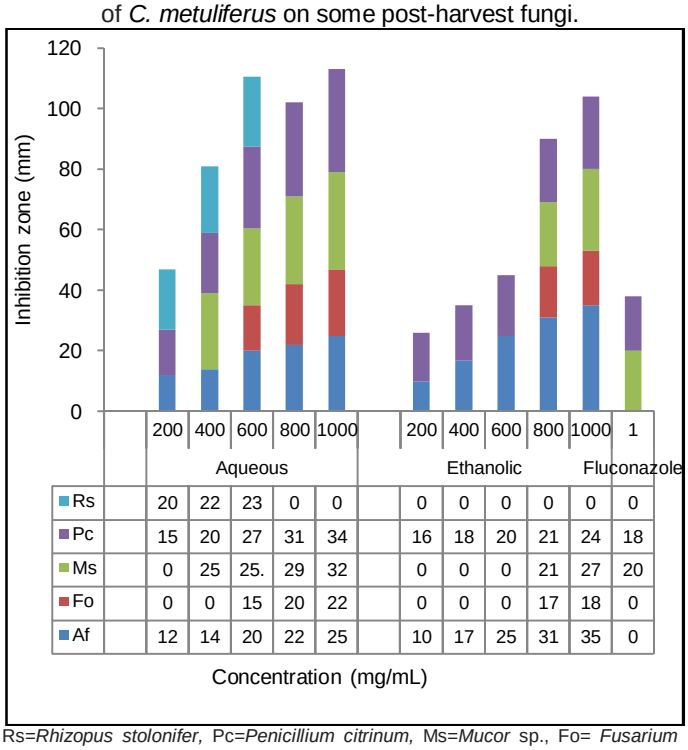
Phytochemical screening of the extracts: Phytochemical screening of extracts of *C. metuliferus* indicated that the compounds present included large quantities of alkaloids and flavonoids. The other components included tannins, carbohydrates, cardiac glycosides and saponins. Steroids and anthraquinones were completely absent (Table 2).

Antifungal screening: The test fungi used in the study demonstrated susceptibility to both the aqueous and ethanolic pulp extract of *Cucumis metuliferus.* The results as shown in Fig. 1 indicated that ethanolic extract had more effect on the *Aspergillus flavus* than the aqueous extract in all the concentrations. Highest effect of 35 mm was observed at 1000 mg/mL, followed by *Mucor* sp. with zone diameter of 27 mm. The least effect was observed on *Fusarium oxysporum* with zone diameter of 18 mm. The various concentrations of 200, 400 and 600 mg/mL had effects only on *A. flavus* and *P. citrinum.* Aqueous extract demonstrated higher activity in all other test fungi than the ethanolic extract. The highest concentration (1000 mg/mL) of the aqueous extract had the highest effect on the test fungi. The highest zone of inhibition of 34 mm was observed on *P. citrinum*, followed by *Mucor* sp. The least zone diameter was recorded for *Fusarium oxysporium* at 22 mm. The effects of the extracts on the test fungi were also found to be greater than that of the standard drug which had effects only on *Mucor* sp. and *Penicillium citrinum*. The details of the results are presented in Fig. 1.

Minimum inhibitory concentration (MIC): The results of MIC of the extracts against the test fungi are presented in Table 3. The MIC for *A. niger* and *P. citrinum* for both aqueous and ethanolic extracts was 12.5 mg/mL while that *of F. oxysporum, Mucor* sp. and *R. stolonifer* was 25 mg/mL.

Minimum fungicidal concentration (MFC): The MFC recorded for *A. niger* and *P. citrinum* was 25 mg/mL while that of *F. oxysporum, P. citrinum* and *R. stolonifer* was 50 mg/mL. The details of the results are shown in Table 4.

Fig. 1. Antifungal effect of aqueous and ethanolic extracts of *C. metuliferus* on some post-harvest fungi.



Rs=*Rhizopus stolonifer*, Pc=*Penicillium citrinum*, Ms=*Mucor* sp., Fo= *Fusarium oxysporum*, Af=*Aspergillus flavus.*

Discussion

The phytochemical test of the crude methanolic stem bark extracts of *C. metuliferus* revealed the presence alkaloids, carbohydrates, flavonoids, saponins and tannins. Steroid and anthraquinones were found to be absent. These results are similar to those of Usman *et al.* (2009) except for anthraquinones which is absent in this species. The alkaloids in the fruit have been found to be much more than that of the leaves as reported by Aliero and Gumi (2012). The author also reported that presence of alkaloids and saponins in the fruit suggests high toxicity and medicinal property which may be responsible for its antimicrobial potential. The bioactive principles have also been known as the defensive mechanism of the plants against different pathogens. Other researchers have also reported similar trends (Hafiza, 2000; Usman *et al.,* 2014).

Hassan *et al.* (2004) reported that these classes (alkaloids, saponins, tannins, anthraquinones, and flavonoids) of compounds are known to have curative activity against several pathogens and therefore could suggest the use locally for the treatment of various illnesses. Flavonoids have been referred to as nature's biological response modifiers due to their ability to modify the body's reaction to allergies, viruses and carcinogens. The flavonoids from *C. metuliferus* have demonstrated antiviral properties (Wannang, 2010). Tannins also decrease bacterial cell proliferation by blocking key enzymes of microbial metabolism (Awosika, 1991). Flavonoids, tannins and saponins were also reported to have inhibitory effect on the growth of *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Candida albicans* thereby supporting the antimicrobial potential of the plant (Manthey, 2000).

The *in vitro* anti-fungal test presented in Fig. 1 showed that the aqueous extract to be more active against *the Fusarium oxysporum, Mucor* sp*., Penicillium citrinum* and *Rhizopus stolonifer*. The ethanolic extract was more effective on *Aspergillus flavus*. These findings are in agreement with that of Penkhae (2005) which *Piper betel, Boesenbergia pandurata* and *Andrographis paniculata* showed activity against *A. oryzae, A. niger* and *Penicillium* species. Aliero and Gumi (2012) reported the inhibitory effect of acetone, methanol and aqueous extracts of fruit of *C. metuliferus on A. niger* and *A. flavus* at different concentrations. The effects of the extracts (aqueous and ethanolic) and the difference between their antimicrobial activities could be explained by the amount of antimicrobial substances present in each form. Alkaloids and flavonoids were found in large quantities in the aqueous than the ethanolic extract (Table 2). Interestingly, the effects of the extracts on the test fungi were found to be greater than that of the standard drug (Fig. 1) which had effects only on *Mucor* sp. and *Penicillium citrinum* with zone diameters of 20 and 18 mm respectively. The aqueous and ethanolic extracts of fruit of *C. metuliferus* inhibited the growth of test fungi with minimum concentrations ranging from 12.5-25 mg/mL. *Aspergillus niger* and *Penicillium citrinum* were the most sensitive to the ethanolic and aqueous extracts requiring the concentration of only 12.5 mg/mL to inhibit their growth, followed by *Fusarium oxysporum*, *Penicillium citrinum* and *Rhizopus stolonifer* which required the concentration of 25 mg/mL to inhibit their growth. Mohanka and Priyanka (2014) reported similar results of MIC of aqueous juice extract of *C. sinensis* to be 12.5 mg/mL for both *Mucor* and *Rhizopus* species. Jenie *et al.* (2001) also reported that the whole extract (mixture of volatile and non-volatile extract) of *Piper betle* inhibited *S. aureus* and *E. coli* at 0.025% (v/v). It is worth mentioning that lower MIC is correlated with higher antimicrobial activity. MIC value of 12.5 mg/mL infers that the extract has great potentiality as potent antifungal agent.

This implies that it can be exploited as preservative in stored food to increase its shelf life. The MFC for both aqueous and ethanolic extracts for *A. flavus* and *P. citrinum* was 25 mg/mL while that of *F. oxysporum*, *Mucor* sp. and *R. stolonifer* was 50 mg/mL indicating that no growth was observed at those concentrations when the fungi were sub-cultured from the MIC tubes.

Conclusion

In conclusion, this study had shown that the aqueous and ethanolic fruit extracts of *Cucumis metuliferus* possess some anti-fungal activity which could be attributed to the various phyto-compounds present in the fruit. The findings of this research work suggest that *C. metuliferus* fruit could be utilized as potential source of natural preservative with the increased interest in food preservation and storage. The aqueous extract especially could be processed in form of nutraceutical foods which can be incorporated in processed foods.

References

1. Adoum, O.A.N.T. and Fatope, M.O. 1997. Bioactivities of some savannah plants in brine Shrimp lethality test in vitro antimicrobial assay. *Int. J. Pharmacogn*. 35(5): 334-337.
2. Aliero, A.A. and Gumi, A.M. 2012. Studies on the germination, chemical composition and antimicrobial properties of *Cucumis metuliferus*. *Ann. Biol. Res*. 3(8): 4059-4064.
3. Amagon, K.I., Wannang, N.N., Iliya, H.A., Lor, L.D. and Chris-Otubor, G.O. 2012. flavonoids extracted from fruit pulp of *Cucumis metuliferus* have antiviral properties. *Brit. J. Pharmaceut. Res.* 2(4): 249-258.
4. Anyanwu, A.A., Jimam, N.S., Dangiwa, D.A., Wannang, N.N. and Falang, K.D. 2014. Protective effects of alkaloids of *Cucumis metuliferus* isolated from the fruit pulp on some vital organs. *J. Phytopharmacol.* 3(4): 259-263.
5. Austwick, P.K.C. 1965. Pathogenicity of *Aspergillus* species. In: Raper KB and Fennell DI (eds). The Genus *Aspergillus*. Williams and Wilkins, Baltimore, MD., pp.82-126.
6. Awosika, F. 1991. Local medicinal plants and health of consumers. *Clin. Pharm. Herbal Med*. 9: 28-29.
7. Barry, A.L. 1980. Procedure for testing antimicrobial agents in agar media. In: *Antibiotics in Laboratory Medicine*. Lorin V (eds.). Williams Wilkins Co., Baltimore, pp.1-23.
8. Bodeker, C., Bodeker, G.O., Grundy, C.K., Burford, G. and Shein, K. 2005. WHO global atlas of traditional, complementary and alternative medicine. World Health Organization, Geneva.
9. Bullerman, L.B., Lieu, F.Y. and Seier, S.A. 1977. Inhibition of growth and aflatoxin production by cinnamon and clove oils, cinnamic aldehyde and eugenol. *J. Food Sci*. 42: 1107-1109.
10. Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 12(4): 564-582.
11. Dabur, R., Singh, H. Chhillar, A.K. Ali, M. and Sharma, G.L. 2004. Antifungal potential of Indian medicinal plants. *Fitoterap.* 75: 389-391.

12. Demma, J., Engidawork, E. and Hellman, B. 2009. Potential genotoxicity of plant extracts used in Ethiopian traditional medicine. *J. Ethnopharmacol.* 122: 136-142.
13. Elgayyar, M., Draughon, F.A., Golden, D.A. and Mount, J.R. 2001. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. *J. Food Prot.* 64: 1019-1024.
14. Evans, W.C. 2009. *Trease and Evans Pharmacognosy* 16th ed. Saunders, Elsevier Ltd. China, p.178.
15. Hafiza, R.E. 2000. Peptides antibiotics. *Lancet.* 349: 418-422.
16. Hassan, M.M., Oyewale, A.O., Amupitan, J.O., Abduallahi, M.S. and Okonkwo, E.M. 2004. Preliminary phytochemical and antibacterial investigation of crude extracts of the root bark of *Detarium microcarpum*. *J. Chem. Soc*. *Niger*. 29: 26-29.
17. Jenie, B.S.L., Andarwulan, N., Puspitasari- Nienaber, N.L. and Nuraida, L. 2001. Antimicrobial activity of *Piper betle* Linn. extract towards foodborne pathogens and spoilage microorganisms. Institute of Food Technologists Annual Meeting. Retrieved from http://ift.confex.com/ift/2001/techprogram/paper_9068.htm, March 13, 2003.
18. Korzeniowska, M. 1990. Pulmonary Aspergillosis caused by *Aspergillus*. *Pneumonol. Pol.* 58: 328-333
19. Kuete, V., Ngameni, B. Mbaveng, A.T. Ngadjui, B. Meyer, J.J. and Lall, N. 2010. Evaluation of flavonoids from *Dorstenia barteri* for their anti-mycobacterial, anti-gonorrheal and anti-reverse transcriptase activities. *Acta Tropica.* 116: 100-104.
20. Legan, J.D. and Voysey, P.A. 1991. Yeast spoilage of bakery products and ingredients. *J. Appl. Bacteriol.* 70: 361-371.
21. Mahesh, B. and Satish, S. 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World J. Agric. Sci*. 4(5): 839-843.
22. Manthey, J.A. 2000. Biological properties of flavonoids pertaining to inflammation. *Microcirculat.* 7(6): 29-34.
23. Mohanka, R. and Priyanka, A.M.J. 2014. Plant extract as natural food preservative against spoilage fungi from processed food. *Int. J. Curr. Microbiol. App. Sci.* 3(8): 91-98.
24. National Committee of Clinical Laboratory Standards (NCCLS). 2004. Performance standards for antimicrobial Susceptibility testing-14th information supplement approval standard M100-S22. NCCLS, Wayne, PA, USA.The committee, pp.20-24.
25. Ogaraku, A.O. 2010. Deleterious effects of fungi on post-harvest crops and their management strategies. Arun A. and Analia E.P. (Eds.). In: *Management of Fungi Plant Pathogens*. CAB international U.K., pp.28-35.
26. Penkhae, W., Piyamat, C., Ngamtip, P. and Sunee, N. 2005. *In vitro* antifungal activity of thai herb and spice extracts against food spoilage fungi. *Kasetsart J. (Nat. Sci.).* 39: 400-405.
27. Puupponen, P.R., Nohynek, L., Meier, C., Kahkonen, M.M. Heinonen, A. and Hopia, K.M. 2001. Antimicrobial properties of phenolic compounds from *Berries. J. Appl. Microbiol*. 90: 494-507.
28. Roodt, V. 1998. *Common wild flowers of the Okavango Delta; medicinal uses and nutritional value.*The Shell Field Guide Series: Part II. Publ. Shell Oil Botswana, p.174.
29. Seyyednejad, S.M. and Motamedi, H. 2010. A review on native medicinal plants in Khuzestan, Iran with antibacterial properties. *Int. J. Pharmacol*. 6(50): 551-560.
30. Sharma, R.C. and Vir, D. 1986. Post-harvest diseases of grapes and studies on their control with benzimidazole derivatives and other fungicides. *Pesticides (Bombay)* 20: 14-15.
31. Skandamis, P., Koutsoumanis, K. Fasseas, K. and Nychas, G.J.E. 2001. Inhibition of oregano essential oil and EDTA on *E. coli* O157:H7. *Ital. J. Food Sci*. 13: 55-65.
32. Sofowora, A. 2008. *Medicinal Plants and Traditional Medicine in Africa*.3rd Ed. Ibadan Spectrum Books Limited. pp.181-204.
33. Tadeg, H., Mohammed, E., Asres, K. and Gebre, T.M. 2005. Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. *J. Ethnopharmacol*. 100: 168-175.
34. Usman, H., Abdulrahman, F.I and Usman, A. 2009. Qualitative phytochemical screening and *in vitro* antimicrobial effects of methanol stem bark extract of *Ficus Thonningii* (Moraceae). *Afri. J. Trad. Complementary Alternat. Med.* 6(3): 289-295.
35. Usman, J.G., Sodipo, O.A. and Sandabe, U.K. 2014. Phytochemical screening and acute toxicity study of *Cucumis metiliferus* E. Mey. Ex. Naudin fruit extract in Cockerels. *Int. J. Phytomed.* 6: 243-247.
36. Wannang, N.N., Kwanashie, H.O. and Ede, S.O. 2010. Antiviral activity of the fruit extracts of *Cucumis metuliferus* E. Meye (Curcubitaceae) in Chicks. *Afri. J. Basic Appl. Sci.* 2(3-4): 89-93.
37. Wilkins-Ellert, M.H., 2004. *Cucumis metuliferus* E.Mey. ex Naudin In: Grubben, G.J.H. & Denton, O.A. (Editors). PROTA 2: Vegetables/Légumes. [CD-Rom].PROTA, Wageningen, Netherlands.
38. World Health Organization 1998. Quality control methods for medicinal plants: Determination of microorganisms. Geneva: WHO, pp.64-73.
39. Zamanian, S., Shahidi, B.G.H. and Saadoun, I. 2005. First report of antibacterial properties of a new strain of *Streptomyces plicatus* (strain 101) against Erwinia carotovora from Iran. *Biotechnol.* 4: 114-120.