Trop J Nat Prod Res, September 2021; 5(9):1650-1655 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Aspergillus flavus, Rhizopus stolonifer and *Mucor* spp. Associated with Deteriorated Mango and Orange Fruits: Occurrence and *In vitro* Susceptibility to Extracts of *Aspilia africana* (Pers.) C. D. Adams (Asteraceae)

Ijato J. Yeni[1]*, Akinjogunla O. Joseph[2], Divine-Anthony Ofon-mbuk[2], Ojo B. Olaposi[3]

[1]*Department of Plant Science and Biotechnology, Faculty of Science, Ekiti State University, P.M.B 5363, Ado-Ekiti, Ekiti State, Nigeria*
[2]*Department of Microbiology, Faculty of Science, University of Uyo, P.M.B.1017, Uyo, Akwa Ibom State, Nigeria*
[3]*The Polytechnic, Ibadan, Department of Biology, Oyo State, Nigeria*

ARTICLE INFO

Article history:
Received 07 May 2021
Revised 02 August 2021
Accepted 23 August 2021
Published online 02 October 2021

ABSTRACT

A large number of fruits are annually lost due to spoilage by microorganisms, most especially rot fungi. This study was undertaken to isolate and identify fungi from spoilt fruits. The fungal isolates from spoilt mango and orange fruits were obtained using standard mycological technique. Bioactivities of aqueous and ethanol extracts of *Aspilia africana* against fungal isolates from spoilt mango and orange fruits were determined using standard protocol. The fungal isolates obtained were *Aspergillus flavus*, *Mucor* spp and *Rhizopus stolonifer*. The leaf extracts of *A. africana* contained varied concentrations of alkaloids, flavonoids, terpene, saponins, tannins, cardiac glycoside, phlobatanins and phenolics. The highest mean inhibition zone diameter (IZ) obtained was 16.8 ± 1.3 mm from the plate containing *R. stolonifer* RSCS-04, while the lowest mean IZ (mm ± S.D) was 9.8 ± 0.2 from the plate containing *R. stolonifer* RSMI-07. Of the 8 fungal isolates tested, 50.0 %, 75.0 %, 100% and 100% were sensitive to 10, 20, 40 and 80 $mgmL^{-1}$ of ethanol leaf extract of *A. africana*, respectively. The highest IZ (14.3 ± 0.3) and lowest IZ (10.0 ± 0.0) for the aqueous leaf extract was observed in the plate containing *A. flavus* AFMI-03 and *A. flavus* AFCS-03. The Regression coefficients (R^2) values of leaf extract of *A. africana* and diameters of zone of inhibition as exhibited by the fungi ranged from 0.5000 to 0.9872. Application of fungicidal plant extracts especially *A. africana* to mitigate fungal pathogenic invasion of fruits should be considered.

***Keywords*:** *A. africana*, Fungi, Extracts, Mango, Orange, Deterioration.

Introduction

Aspilia africana, hemorrhage plant, is one of about 50 species of the genus *Aspilia* in the family of Asteraceae.[1] *Aspilia africana* is widely distributed across tropical Africa, occurring on waste land of the savanna and forest zone**.** The plant has been widely reported to have many biological activities and is widely used in African traditional folkloric medicine for treatment of several diseases such as gonorrhea, subcutaneous parasitic infection, abdominal pains, intestinal worms and backache.[2,3] Its activities against *Plasmodium falciparum*; lactation enhancement capacity of *A. africana* root decoction upon administration on nursing mothers; haemostatic, analgesic and sedative activities have been reported.[4] Varied chemical compositions of the plants have been reported due to diversity of ecological conditions.

Orange (*Citrus sinensis* L.) is a berry citrus fruit that ranges widely in size and diameter, shape, colour and juice quality, belonging to the family Rutaceae and genera *Citrus.*[5]

The orange fruit consists of vitamins, calcium, iron, magnesium, zinc, phosphorus, potassium, flavonoids and terpenes.[6]

*Corresponding author. E mail: considerureternity@gmail.com
Tel: 08146144519

Citation: Ijato JY, Akinjogunla OJ, Divine-Anthony O, Ojo BO. *Aspergillus flavus, Rhizopus stolonifer* and *Mucor* spp. Associated with Deteriorated Mango and Orange Fruits: Occurrence and *In vitro* Susceptibility to Extracts of *Aspilia africana* (Pers.) C. D. Adams (Asteraceae). Trop J Nat Prod Res. 2021; 5(9):1650-1655. doi.org/10.26538/tjnpr/v5i9.20

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Orange fruits aid in preventing cold and recurrent ear infection,[6] proper functioning of immune system and treatment of arthritis, asthma, Alzheimer's disease, Parkinson's disease, macular degeneration, diabetes mellitus and gallstones.[7] The fiber in orange reduces a high cholesterol level thereby helping to prevent atherosclerosis. The fibers also help in keeping blood sugar levels under control, which explains why orange can be a healthy snack for diabetic patients.[8]

Mango (*Mangifera indica* L.) is a species of flowering plants belonging to the family *Anacardiaceae* and order *Sapindales.*[9] Mango fruits are highly perishable and contain water, proteins, carbohydrates, fats, sugars, dietary fibers, calcium, copper, phosphorus and vitamins (vitamins E, B_1, B_2, B_3, B_5, B_6, and B_9).[10] Depending on cultivar, mango fruit varies in size, shape, sweetness, skin colour and fresh colour which may be pale yellow, gold or orange. Mango fruits help in controlling high cholesterol level and aids in maintaining the alkali reserve of the body. It has also been reported that mango fruits aid good digestion and improve eyesight.[9]

The association among fruits, microorganisms and human has been long, interesting and developed before recorded history.[11] Fungi colonize fruits as substrate for their growth and the genera *Candida*, *Saccharomyces*, *Penicillium*, *Aspergillus*, *Mucor*, *Cladosporium*, *Fusarium*, *Talaromyces* and *Neosartorya* are the most frequently isolated microbes from fresh fruits.[12] During the sequence of post-harvest fruit handling such as transport, storage and marketing before final consumption, microorganisms can affect the fruit quality as well as human health. Fungi as agents of deterioration can invade and cause spoilage of fruits after tissues of the fruit have been damaged by some physical and physiological causes.[11] This study was aimed at evaluating the antifungal activity of leaf extracts of *Aspilia africana* on fungal isolates associated with deteriorated orange and mango fruits.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Materials and Methods

Source and Collection of Fruit Samples

Samples of infested fruits of mango and orange were obtained from Akpan Andem and Use-Offot markets in Uyo metropolis, Akwa Ibom State. The rotten fruit samples obtained were separately placed in sterile polyethylene bags, labeled appropriately and transported to Microbiology Laboratory for mycological analyses.

Isolation of Fungal Isolates from Rotten Fruits

The isolation technique used by Dashwood *et al.*[13] and Balali *et al*. [14] was employed in this study. The infested mango and orange fruit samples were separately rinsed thrice in distilled water and surface sterilized with cotton wool soaked in 70% ethanol. Sterile scalpel and forceps were used to cut small sections of the tissue showing both the rotten portion and adjoining healthy tissue (3 mm diameter) and was aseptically plated on solidified potato dextrose agar (PDA) containing streptomycin (10 µg/mL) to prevent bacterial growth. The inoculated plates were aerobically incubated at room temperature (28 ± 2^{o}C) for 5 days. After incubation, the individual hypha tips of the emerging fungal colonies in the plates were aseptically picked, sub-cultured onto a plate of freshly prepared sterile PDA and incubated at room temperature (28 ± 2^{o}C) for 5 days. Discrete fungal colonies were stocked on PDA slant, incubated at 28 ± 2^{o}C for 5 days and stored in the refrigerator.

Characterization and Identification of Fungal Isolates

The fungal isolates were characterized and identified microscopically and macroscopically based on their colonial growth pattern, conidia, morphology and pigmentation.[15]. Drops of lacto-phenol cotton blue were placed at the centre of clean grease-free slides. A portion of mycelium of fungal isolate was placed in cotton blue-in-lactophenol on a slide, emulsified and cover slip was placed at the centre of the slide prior to direct observation under a light microscope. The structure of the mycelium, spore structure and fruiting bodies were appropriately identified.[16]

Sources of Medicinal Plants

The fresh *Aspilia africana* leaves were obtained in October 2020 from their natural habitats in Akwa Ibom State and the identity of this plant was authenticated with voucher specimen of the plant (EBC/2020/Asp) deposited in the herbarium unit of Department of Botany and Ecological Studies, University of Uyo, Uyo. Thereafter, the plant was taken to Pharmacognosy and Natural Medicine Laboratory, Faculty of Pharmacy, University of Uyo for processing. The *A. africana* leaves were washed three times in running tap and rinsed with distilled water to remove extraneous matters. The plant leaves were air-dried, pulverized and stored in air-tight polythene bag.

Preparation of Plant Extract

Each of pulverized *A. africana* (3 kg) was exhaustively extracted using the method of Kage *et al.*, [17] After evaporation, the extract was weighed and preserved in a brown bottle. The graded concentrations (10 mgmL^{-1}, 20 mgmL^{-1}, 40 mgmL^{-1} and 80 mgmL^{-1}) of the extracts were prepared using 10% Dimethyl Sulphoxide (DMSO, Sigma, USA), shaken vigorously to obtain a homogenous mixture and the extracts were preserved in a refrigerator at 4^{o}C.

In vitro Antifungal Susceptibility Testing of Plant Extracts

The aqueous and ethanol extracts of *A. africana* were assayed for their antifungal activities by disc diffusion method.[18] Sterilized PDA was dispensed into sterile Petri dishes. Sterile filter paper discs (6 mm diameter) were incorporated with each ethanol, aqueous extracts solution of graded concentrations (10 mgmL^{-1}, 20 mgmL^{-1}, 40 mgmL^{-1} and 80 mgmL^{-1}) and these discs were placed on agar plates which had been previously inoculated with fungal isolates. Thereafter, the plates were incubated at room temperature for 3 days. Treatment with concentration of each extract was replicated thrice and the mean zone of inhibition diameter (in millimeters) was determined in each case.

Phytochemical Screening of A. africana extracts

The phytochemical compositions of the plant extracts were assayed according to the methods described by Trease and Evans.[19]

Statistical Analysis

The Statistical Package for Social Sciences (IBM SPSS Version 22.0. Armonk, NY: IBM Corp.) was used for data analysis. The mean and standard deviation of zones of inhibition were determined**.** The relationship between different concentrations of extracts and the overall antifungal activity was assessed as diameters of zones of inhibition with regard to the fungal isolates as determined by linear regression analysis and were statistically significant at p-value < 0.05.

Results and Discussion

The fungal isolates obtained from the deteriorated orange and mango fruits using their colonial and morphological characteristics (shape, type of soma, nature of hyphae, pseudo-mycelium and asexual reproductive spore) were *Aspergillus flavus, Mucor* spp and *Rhizopus stolonifer* from *M. indica* (mango) and *A. flavus* and *R. stolonifer* from *C. sinensis* (orange) (Table 1).

The number and percentage occurrences of fungal isolates in *M. indica* was *A. flavus* 1 (10.0%), *Mucor* spp 1 (10.0%) and *R. stolonifer* 2 (20.0%). The two fungal isolates from *C. sinensis* were *A. flavus* 3(30.0 %) and *R. stolonifer* 1 (10.0%) (Table 2).

The phytochemical analyses of aqueous and ethanol leaf extracts of *A. africana* are presented in Table 3. The aqueous leaf extracts of *A. africana* contained alkaloids (+++), flavonoids (++), terpenes (+), saponins (+), tannins (+), cardiac glycoside (+), phlobatanins (+) and phenolics (+) (Table 3). The ethanol leaf extracts of *A. africana* contained alkaloids in a very high concentration (+++); flavonoids, saponins and cardiac glycoside were detected in moderately high concentration (++), while terpenes, tannins, deoxy sugar, phlobatanins and phenolics were detected in low concentration (+) (Table 3).

Table 1: Colonial and Morphological Characteristics of Fungal Isolates from Deteriorated Orange and Mango Fruits

Colony Colour	Colony Shape	Type of Soma	Nature of Hyphae	Pseudo-mycelium	Asexual Reproductive Spore	Probable Fungal Isolates
Yellow fluffy mycelia and some black sporangiospores	Circular	Filamentous	Thick septate hyphae	Foot cell	*Globose conidia*	*Aspergillus flavus*
Colonies light grey, mycelia growing rapidly and filling the Petri dish with dense/fluffy cottony mycelium	Circular	Filamentous	Aseptate	-	Sporangia globose with some flattened bases, contained many spores	*Rhizopus stolonifer*
Cottony to fluffy white to yellow, dark green with sporangia	Circular	Filamentous	Aseptate	Foot cell	Smooth and regular sporangium	*Mucor* spp

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The results of antifungal activities of the varied concentrations of extracts of *A. africana* on *A. flavus*, *Mucor* spp. and *R. stolonifer* are presented in Tables 4 and 5. The antifungal activities of ethanol leaf extract of *A. africana* are presented in Table 4. The highest mean inhibitory zone diameter (IZ) obtained was 16.8 ± 1.3 mm from the plate containing *R. stolonifer* RSCS-04, while the lowest mean IZ (mm ± S.D) was 9.8 ± 0.2 from the plate containing *R. stolonifer* RSMI-07. Of the 8 fungal isolates tested, 4/8 (50.0%), 6/8 (75.0 %), 8/8 (100%) and 8/8 (100%) were sensitive to 10, 20, 40 and 80 $mgmL^{-1}$ of ethanol leaf extract of *A. africana*, respectively (Table 4). The antifungal activities of aqueous leaf extract of *A. africana* are shown in Table 5. The highest and lowest inhibitory zone was observed in the plate containing *A. flavus* AFMI-03 and *A. flavus* AFCS-03 with the corresponding mean ± S.D of 14.3 ± 0.3 mm and 10.0 ± 0.0 mm, respectively. Of the 8 fungal isolates tested, 3/8 (37.5 %), 4/8 (50.0 %), 8/8 (100 %) and 8/8 (100 %) were sensitive to 10, 20, 40 and 80 $mgmL^{-1}$ of aqueous leaf extract of *A. africana*, respectively (Table 5).

Regression coefficients (R^2) between different concentrations of leaf extract of *A. africana* and diameters of zone of inhibition as exhibited by fungal isolates from deteriorated orange and mango fruits are presented in Table 6 and Figures 1 to 8. The R^2 values of ethanol leaf extract of *A. africana* and diameters of zone of inhibition as exhibited by the fungal isolates ranged from 0.5000 to 0.9335, while the R^2 values of aqueous leaf extract of *A. africana* and diameters of zone of inhibition as exhibited by the fungal isolates ranged from 0.6220 to 0.9872 (Table 6).

Spoilage microorganisms are introduced into the crop via the seed, during crop growth in the field; during harvesting, post-harvest handling, storage, distribution, loading and offloading.[20] The fruits are usually displayed on benches and in baskets for prospective customers in the open markets until sold, thereby exposing them to further microbial infection. [21] The identified fungal isolates associated with spoilt fruits in this study were *A. flavus*, *Rhizopus* spp. and *Mucor* spp. Our finding was in conformity with results of Baiyewu *et al*. [22] and Chukwuka *et al*.[21] who reported these fungi in spoilt *C. papaya* fruits in Southwest, Nigeria. The isolation of fungal isolates in the spoilt *C. sinensis* and *M. indica* fruits in this study showed that spoilt fruits could harbour some pathogenic fungi which may cause diseases in man and animals. The isolation of *A. flavus* in both *M. indica* and *C. sinensis* fruits in this study corroborated the reports of Okereke *et al*.[23] who obtained *A. flavus* in deteriorated *M. indica* and *C. sinensis* fruits. Microbial spoilage in fruit varies not only with the kind of fruits but also to some extent with the varieties.[11] The presence of cut and damaged surfaces provides an opportunity for contamination and growth of microorganisms and ingress into plant tissues. Microbial spoilage may be due to pathogens acting on stems, leaves, flowers or root of the plants on the fruit or other special parts used as food.[22] Saprophytic organisms are secondary invaders that may enter healthy fruits and they have been reported to be involved in spoilage of fruits.[24]

The results of phytochemical analysis showed that *A. africana* contained alkaloids, flavonoids, terpenes, saponins, tannins, cardiac glycoside, deoxy sugar, phenolic and phlobatanins. The detection of these phytochemical constituents in *A. africana* in this study confirmed the findings of Ekaiko *et al*.[25] who reported flavonoids, terpenes, saponins, tannins and cardiac glycoside as the phytochemical constituents present in *A. africana*. In this study, extracts of *A. africana* displayed antifungal activity against fungal isolates from spoilt fruits and this confirmed the findings of Johnson *et al.* [26] on the antimicrobial activity of compounds isolated from the leaves of *A. africana*. In this study, the test fungal isolates were significantly more affected by the ethanol extract of *A. africana* as exhibited by wider zones of inhibition than aqueous extract of *A. africana*. This may be attributed to variations in the dissolution capacity of the different solvents which in turn affected the degree of phytochemicals extracted.

Several studies have attributed the physiological activity, medicinal and antimicrobial properties of *A. africana* to abundant bioactive secondary metabolites.[27] Moderately high concentrations of flavonoids and tannins observed in *A. africana* may be responsible for its antifungal efficacies on spoilage pathogens. Flavonoids are hydroxylated phenolic substances that are synthesized by plants in response to microbial infection,[28] and the activity of flavonoids is possibly due to their ability to disrupt the microbial membrane.[29]

Table 2: Occurrence of Fungal Isolates in Deteriorated Orange and Mango Fruits

Isolates	No (%) Occurrence in Deteriorated Fruits		
	M. indica	***C. sinensis***	**Total**
A. flavus	1 (10.0)	3(10.0)	4
Mucor spp	1 (10.0)	0 (0.0)	1
R. stolonifera	2 (20.0)	1 (10.0)	3
Total	4	4	8

Table 3: Phytochemical Analyses of Aqueous and Ethanol Leaf Extracts of *A. africana*

Plant constituents	Inference	
	Ethanol Extracts	**Aqueous Extracts**
Alkanols	+	+
Flavonoids	+	+
Terpenes	+	+
Saponins	+	+
Tannins	+	+
Steroids	ND	ND
Cardiac glycoside	++	+
Deoxy sugar	+	-
Phlobatanins	+	+
Phenolics	+	+

Key: ND: Not Detected.

Table 4: Antifungal Activities of Ethanol Leaf Extract of *Aspillia africana* on Fungal Isolates

Fungal Isolates	Code of Isolates	Zone of Inhibition (mm ± S.D)				10% DMSO
		10 mg/mL	20 mg/mL	40 mg/mL	80 mg/mL	NZ
A. flavus	AFCS-03	12.6 ± 0.2	13.4 ± 0.5	15.2 ± 1.1	15.7 ± 0.5	NZ
	AFCS-03	10.2 ± 0.2	11.5 ± 0.5	13.7 ± 0.2	15.0 ± 1.0	NZ
	AFCS-05	NZ	NZ	9.9 ± 0.1	11.2 ± 0.2	NZ
	AFCS-10	NZ	12.1 ± 0.1	14.0 ± 0.5	16.6 ± 0.3	NZ
Mucor spp	MCMI-06	NZ	NZ	10.5 ± 0.0	13.9 ± 0.1	NZ
R. stolonifer	RSMI-07	9.8 ± 0.2	11.1 ± 0.1	13.2 ± 0.2	13.9 ± 0.1	NZ
	RSMI-09	NZ	9.5 ± 0.2	10.8 ± 0.1	11.6 ± 0.3	NZ
	RSC-04	11.7 ± 0.2	13.3 ± 0.2	15.0 ± 1.0	16.8 ± 1.3	NZ

Keys: NZ: No Zone of inhibition; mm: mean: S.D: Standard Deviation: DMSO: Dimethyl Sulphoxide; Each value represents the mean of three replicates and standard deviation.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 5: Antifungal Activities of Aqueous Leaf Extract of *A. africana* on Fungal Isolates

Fungal Isolates	Code of Isolates	Zone of Inhibition (mm ± S.D)				10% DMSO
		10 mg/mL	20 mg/mL	40 mg/mL	80 mg/mL	NZ
A. flavus	AFCS-03	11.2 ± 0.2	12.7 ± 0.2	14.0 ± 1.0	14.3 ± 0.3	NZ
	AFCS-03	10.0 ± 0.0	10.8 ± 0.2	11.4 ± 0.1	13.7 ± 0.2	NZ
	AFCS-05	NZ	NZ	8.0 ± 0.0	8.9 ± 0.0	NZ
	AFCS-10	NZ	NZ	8.9 ± 0.1	11.1 ± 0.1	NZ
Mucor spp	MCMI-06	NZ	NZ	10.0 ± 0.2	12.4 ± 0.4	NZ
R. stolonifer	RSMI-07	9.8 ± 0.2	8.6 ± 0.1	10.2 ± 0.2	12.2 ± 0.2	NZ
	RSMI-09	NZ	NZ	9.6 ± 0.1	11.0 ± 0.0	NZ
	RSC-04	11.7 ± 0.2	12.7 ± 0.2	13.5 ± 0.5	14.1 ± 1.1	NZ

Keys: NZ: No zone of inhibition; mm: mean; S.D: Standard Deviation; DMSO: Dimethyl Sulphoxide;
Each value represents the mean of three replicates and standard deviation.

Table 6: Regression Coefficients between Different Concentrations of Leaf Extract of *A. africana* and Diameters of Zone of Inhibition Exhibited by Fungal Isolates

Fungal Isolates	Code of Isolates	Regression (R^2)	
		Ethanol Extracts	Aqueous Extracts
A. flavus	AFMI-03	0.8328	0.7325
	AFCS-03	0.9008	0.9872
	AFCS-05	0.7777	0.8377
	AFCS-010	0.5997	0.8288
Mucor spp	MCMI-06	0.8398	0.8259
R. stolonifer	RSM1-07	0.8322	0.6220
	RSM1-09	0.5000	0.7848
	RSCS-04	0.9335	0.6958

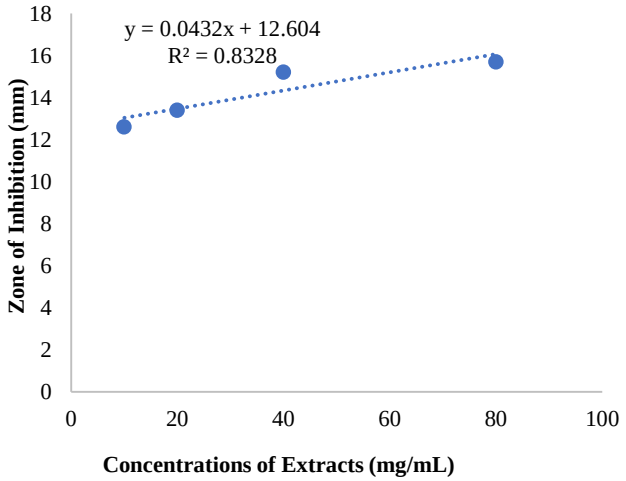

Figure 1: Relationship between Conc of Ethanol Extract of *A. africana* and Inhibitory Zones as Exhibited by AFMI-03

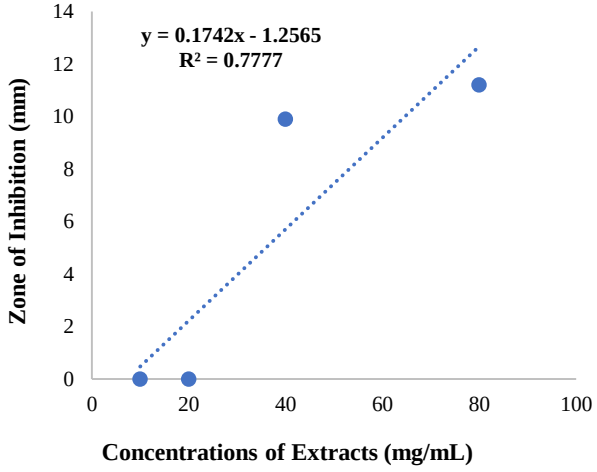

Figure 2: Relationship between Conc. of Ethanol Extract of *A. africana* and Inhibitory Zones as Exhibited by AFCS-05

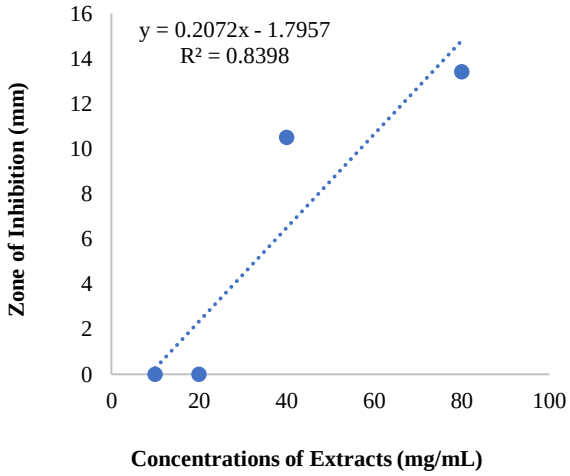

Figure 3: Relationship between Conc of Ethanol Extract of *A. africana* and Inhibitory Zones as Exhibited by MCMI-06

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

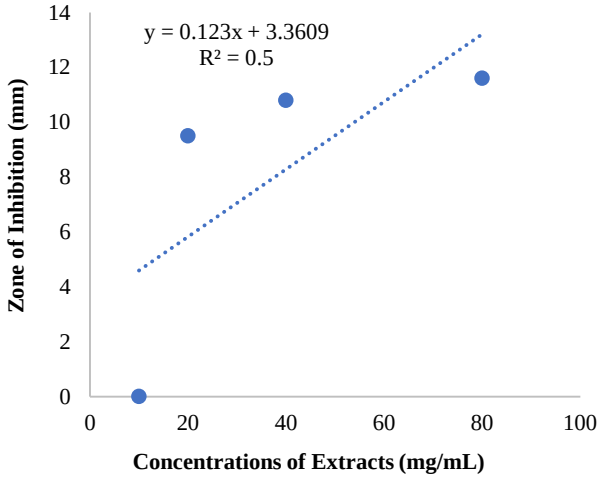

Figure 4: Relationship between Conc of Ethanol Extract of *A. africana* and Inhibitory Zones as Exhibited by RSMI-09

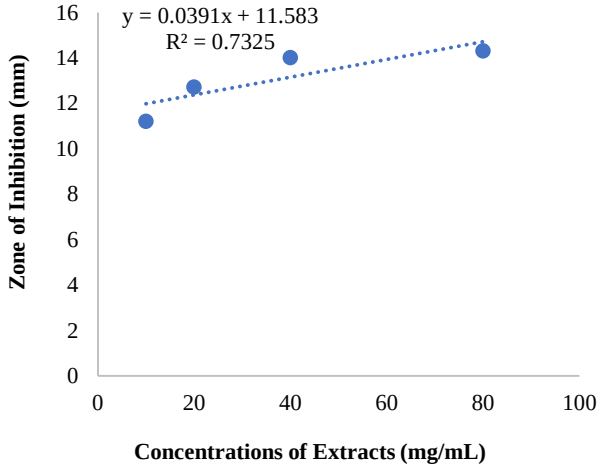

Figure 5: Relationship between Conc of Aqueous Extract of *A. africana* and Inhibitory Zones as Exhibited by AFMI-03

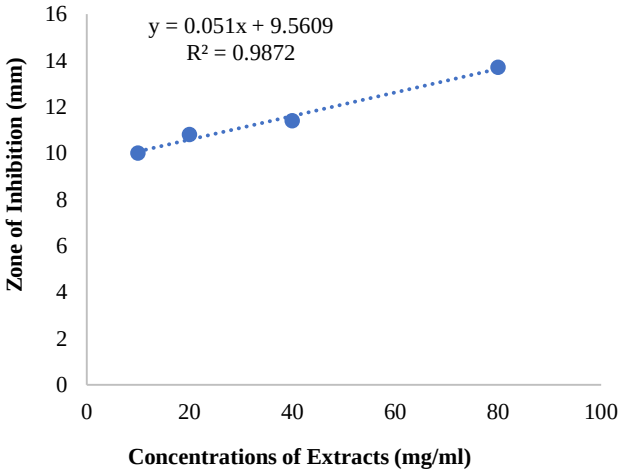

Figure 6: Relationship between Conc of Aqueous Extract of *A. africana* and Inhibitory Zones as Exhibited by AFCS-03

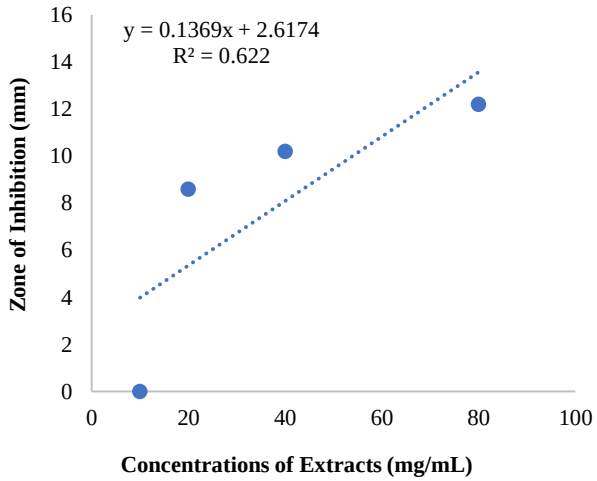

Figure 7: Relationship between Conc of Aqueous Extract of *A. africana* and Inhibitory Zones as Exhibited by RSMI-03

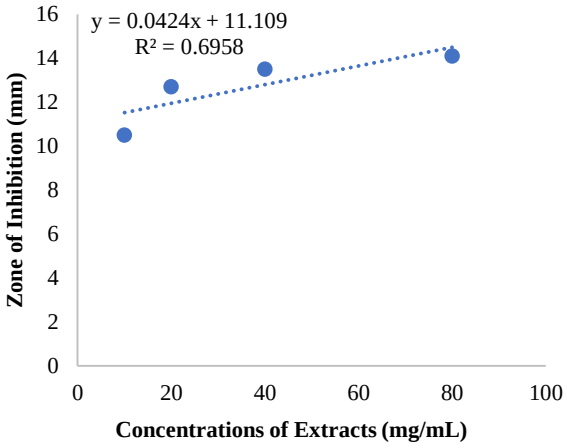

Figure 8: Relationship between Conc of Aqueous Extract of *A. africana* and Inhibitory Zones as Exhibited by RSCS-03

Conclusion

This study has therefore shown the fungal isolates associated with the spoilage of *M. indica* and *C. sinensis.* The presence of some secondary metabolites in aqueous and ethanol extracts of *A. africana* might have contributed to its efficacies against the fungal isolates associated with these spoilt fruits.

Conflicts of Interest

The authors declare no conflict of Interest.

Authors' Declaration

The authors hereby declare that the presented work in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgement

The authors would like to thank all support staff and students who participated in this study.

References

1. Komakech R, Matsabisa MG, Kang Y. The wound healing potential of *Aspilia africana* (Pers.) C. D. Adams (Asteraceae). eCAM. 2019; 1-12: Article ID 7957860.
2. Johns T, Kokwaro JO, Kimanani EK. Herbal remedies of the Luo of Siaya District, Kenya: Establishing quantitative criteria for consensus. Econ Bot. 1990; 44:369-381.
3. Masinde P.S. Medicinal plants of the Marachi people of Kenya. Proceedings of the 14th AETFAT Congress Wageningen, August 22-27, 1996, The Netherlands,1996; pp: 747-750.
4. Offulla AV, Rukunga GM, Chege GM, Kiarie F, Muthaura CN, Githure JI, Kofi-Tse WM. Antimalarial activity of fractions isolated from *Albizia gummifera* and *Aspilia mossambicensis* crude extracts. Afr J Health Sci.1996; 3: 44- 46.
5. Parle M and Chaturvedi D. Orange: range of benefits. Int Res J Pharm. 2012; 3(7):59-63.
6. Ihrig M. Qualitaskantrolle von subem orangeenschalnol. Pharm Zeitung**.** 1995; 140:2350- 2353.
7. Simon JA, Hudes ES, Perez-Perez GD. Relation of serum ascorbic acid to *H. pylori* serology in US adults. The 3rd National Health and Nutrition Examination Survey. J Am Coll Nutr. 2003; 22:283-289.
8. Kurowska EM and Manthey JA. Hypolipidemic effects and absorption of citrus polymethoxylated flavones in hamsters with diet induced hypercholesterolemia. J Agric Food Chem. 2004; 19: 2879-2886.
9. Khan N, Khushtar M, Nesar A, Noorul H, Khan Z, Sahar I, Ahmad A, Zishan M. Nutritional importance and pharmacological activity of *Mangifera indica.* World J Pharm Pharm Sci. 2017; 6(5):258-273.
10. Berger MM. Vitamins and trace elements. Pract Asp Suppl Nut. 2006; 22:952-955.
11. Divine-Anthony O, Okwulehie CI, Akinjogunla OJ. Aflatoxin and ochratoxin producing potentials of moulds associated with spoilage of fruits marketed in Akwa Ibom State. World J App Sci Tech. 2018; 10(1):43-49.
12. Kamal RA, Dhiman R, Aggarwal NK, Kumar V, Manpreeet KM. Microbes associated with freshly prepared juices of citrus and carrots. Int J Food Sci. 2014; 1-7, Article ID 408085.
13. Dashwood EP, Fox RA, Perry DA. Effect of inoculum source on root and tuber infection by potato blemish disease fungi. J Plant Pathol. 1992; 1 41:215-223.
14. Balali GR, Neate SM, Scott ES, Whisson DL, Wicks TJ. Anastomosis group and pathogenicity of isolates of *Rhizoctonia solani* from potato crops in South Australia. Plant Pathol. 1995; 44:1050-1057.
15. Oyeleke SB and Manga SB. Essential of Laboratory Practicals in Microbiology. 1st Edn.,Tobest Publisher, Niger State, Nigeria, 2008; pp: 36-58.
16. Barnett HL and Hunter BB. Illustrated Genera of Imperfect Fungi. 4th edn, McMillian, New York. 1987. 218 p.
17. Kage DN, Seetharam YN, Malashetty B. *In-vitro* antibacterial property and phytochemical profile of *Trichosanthus cucumerina* L var *Cucumerina*. Adv Nat Appl Sci. 2009; 3 (3):438-441.
18. Somchit MN, Mutalib AR, Ahmad Z, Sulaiman MR, Norli S. *In vitro* antifungal activity of *Cassia tora* L. J Trop Med Plants. 2004; 5(1):15-20.
19. Trease GE and Evans WC. Trease and Evans Pharmacognosy 14th edition W.B Sunder company limited, London. 1996; Pp 191-293.
20. Gadgile DP and Chavan AM. Impact of temperature and relative humidity on development of *Aspergillus flavus* rot of mango fruit. Sci Technol. 2010; 3:48-49.
21. Chukwuka KS, Okonko IO, Adekunle AA. Microbial ecology of organisms causing pawpaw (*Carica papaya* L.) fruit decaying, Oyo State, Nigeria. Am Eurasian J Toxicol Sci. 2010; 2:43-50.
22. Baiyewu RA, Amusa NA, Ayoola OA, Babalola OO. Survey of the postharvest diseases and aflatoxin contamination of marketed Pawpaw fruit (*Carica papaya* L.) in South Western Nigeria. Afr J Agric Res. 2007; 2(4):178-181.
23. Okereke VC, Godwin-Egein MI, Arinze AE. Assessment of postharvest rot of mango at different stages of market in Port Harcourt, Nigeria. Int J Curr Res. 2010; 11:6-10.
24. Lewis RA. CRC Dictionary of Agricultural Sciences. Boca Raton, FL: CRC Press. 2002; pp160
25. Ekaiko MU, Arinze AG, Iwe CU. Phytochemical constituents and antimicrobial potency of *Aspilia africana*. Int J Life Sci Res. 2016; 4(1):9-14.
26. Johnson EC, Mohammad IB, Mohammed IS, Olorunfemi AE, Hammed O, Oladimeji A, Emmanuel I, Etim A, Aniefiok SU. Antimicrobial activity of compounds isolated from the leaves of *A. africana* (Pers.). Afr J Pharmacol Ther. 2016; 5(3):149-154.
27. Okwuonu U, Baxter-Grillo DC, Njoya H, Iyemene PT. Proximate and elemental constituents of *Aspilia africana* (Wild sunflower) flowers. J Med Plants Stud. 2017; 5(4):22-27.
28. Akinjogunla OJ, Etok CA, Oshoma CE. Preliminary phytochemistry and *in- vitro* antibacterial efficacy of hydro-ethanolic leaf extracts of *Psidium guajava*: The potential of urinary tract infection treatment. Bio Bulletin. 2011; 5:329-336.
29. Akinjogunla OJ, Adenugba IT, Jumbo OM. *In-vitro* antibacterial evaluation of ethanolic stem crude extracts of *Anacardium occidentale* Linn. (Anacardiaceae) on *Streptococcus mutans* associated with dental caries. SJM. 2012; 1(3):71-81.