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QUANTITATIVE ESTIMATION OF METABOLITES AND ANTIFUNGAL EFFICACY OF LEAF EXTRACTS *ASPILIA AFRICANA* ON CUCUMBER AND PAWPAW FRUIT SPOILAGE FUNGI

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ABSTRACT
Several reports have shown that about 25% of harvested fruits globally are lost to spoilage by microorganisms. The study determined the quantitative metabolites and bioactivities of *Aspilia africana* extracts on fungi from spoiled *Cucumis sativus* and *Carica papaya* fruits. The fungi from spoiled *C. sativus* and *C. papaya* fruits were obtained using mycological techniques. The quantitative metabolites and bioactivities of the aqueous (ALEAA) and ethanol (ELEAA) extracts of *A. africana* were determined using the standard protocol and disc diffusion technique, respectively. The fungal genera obtained were Aspergillus, Penicillium, Mucor, Fusarium, and Rhizopus. There were variations in percentage yields, physical appearances, and pH of the extracts. The results of quantitative estimation of metabolites showed that ALEAA had mean protein, carbohydrate, and lipid contents of 15.36 ± 0.32%, 60.97 ± 1.14%, and 6.66 ± 0.04%, respectively. Alkaloids showed a positive correlation with protein (r = 0.2028) and carbohydrate (r = 0.421), and a negative correlation with lipid (r = -0.6556) at $p < 0.05$. The ELEAA exhibited more inhibitory effects on test fungal isolates, with mean zones of inhibition (IZs) ranging from 9.3 ± 0.1 to 18.8 ± 0.3 mm, than the ALEAA, with mean IZs ranging between 9.4 ± 0.4 and 16.0 ± 1.0 mm. The R coefficients of the extracts and IZs as exhibited by the fungi ranged from 0.5985 to 0.9936. The results have revealed quantitative metabolites and antifungal activities of the extract and also provided rationale for its utilization as a preservative for fruits against spoilage by fungi.

Keywords: Metabolites, *Aspilia africana*, Bioactivities, *Cucumis sativus, Carica papaya*.

INTRODUCTION
Aspilia africana (Pers.) C. D. Adams, a semi woody and haemorrhage plant, belongs to the family Asteraceae (Komakech *et al*., 2019). The *A. africana* is widely distributed across West Africa, and is found on waste land of the savannah and forest zones (Abi and Onuoha, 2011; Ijato *et al*., 2021). The heights of this perennial herb range between 60 and 300 cm, depending on the amount of rainfall and soil fertility. In Nigeria, *A. africana* is known as 'Yunyun' in Yoruba, 'Orangila' in Igbo, 'Tozalin' in Hausa, 'Edemedong' in Efik and 'Ebe-ugbo' by the Esans (Abi and Onuoha, 2011; Ajeigbe *et al*., 2013). Similarly, in some African countries, *A. africana* is known as 'Nyana' in Kissi (Sierra Leone), 'Fofo' in Akan-akyem (Ghana), 'Mbnaso' in Kpe (Cameroon) and 'Winnih' in Mano (Liberia) (Okello and Kang, 2019). Ethnomedicinally, *A. africana* has been widely reported to have biological activities (Ijato *et al*., 2021) and is widely used for treatment of diarrhoea, malaria, tuberculosis, gastric ulcers, febrile headaches (Sherah *et al*., 2014), gonorrhea (Okello and Kang, 2019).
Cucumis sativus L. (Cucumber) of gourd family Cucurbitaceae and order Cucurbitales is a monoecious and creeping vine that bears cylindrical fruits plants (Bello *et al*., 2014). The distinctive cultivars of *C. sativus* fruit is roughly cylindrical, but elongated with tapered ends and may be 62 cm long and 10 cm in diameter (Zhang *et al*., 2019). Cucumber plants originated from the Asia continents (Abulude and Adeleke, 2010) and are now cultivated in both temperate and tropical environment (Mortimore, 2015). In Nigeria, *C. sativus* is cultivated in Northern central during both rainy and dry seasons using irrigation (Ishaya *et al*., 2019). The *C. sativus* contains vitamins (Vit A, B_1, B_2 and B_3), proteins, fats, carbohydrates, calcium, magnesium, iron, potassium, sodium, phosphorus and zinc (Bello *et al*., 2014).

Carica papaya L. (Pawpaw) is a succulent, 2-10 m tall, soft-wooded perennial, and herbaceous plant belonging to Carcicaceae Family. The *C. papaya* originated from area between Mexico and Central America (Menzel, 1994), and is currently grown in all tropical countries and many sub-tropical regions of the world. *Carica papaya* is nutritionally rich in antioxidants, Na, K, Ca, Mg, P, Fe, Cu, Zn and Mn (Krishnan *et al*., 2012). In addition, ripe *C. papaya* fruit is a source of carotenoids, pantothenic acid, water, sugar, protein, ash and vitamins (Adetuyi *et al*., 2008). The *C. papaya* fruit can be freshly eaten or cooked, and used in the preparation of jellies, juice and jams. The mild laxative action of *C. papaya* and its medicinal uses against worms and ulcer have been reported (Baiyewu, 1994). 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 (Divine-Anthony *et al*., 2018). Spoilage microorganisms such as genera *Penicillium, Aspergillus, Mucor, Fusarium* and *Talaromyces* may invade fruits during growth and as well during post-harvest handling (Ijato *et al*., 2021). This study determined the quantitative metabolites and antifungal potency of *A. africana* on fungal pathogens associated with post-harvest spoilage of *C. sativus* and *C. papaya* fruits.

MATERIALS AND METHODS

Sample collection
Spoilt fruits of *C. papaya* (pawpaw) and *C. sativus* (cucumber) were randomly purchased from the fruit sellers at two different markets

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in Uyo, Akwa Ibom State. The spoilt fruit samples purchased were separately packaged into different sterile polyethylene bags, labelled appropriately, and were transported to the Microbiology Laboratory for mycological analysis.

Isolation of fungal isolates from spoilt fruits

A total of 20 randomly selected spoilt fruits (*C. papaya,* n=10) and (*C. sativus,* n=10) were examined. The technique employed by Balali *et al*. (1995) was used for isolation of fungi from the spoilt fruits. The spoilt pawpaw and cucumber fruit samples were rinsed thrice with distilled water (dH_2O) and surface sterilized with cotton wool soaked with 70% (v/v) ethanol for 2 min. Surface sterilized scalpel and forceps were used to cut a small section of the tissue showing both the spoilt / rotten portion and adjoining healthy tissue and was aseptically plated on Potato Dextrose Agar (PDA) containing Streptomycin (10 µg/mL). The inoculated PDA plates were incubated at room temp of 28 ± 2°C for 5 days. The individual hypha tips of different fungal colonies that emerged in the PDA plates were picked, sub-cultured onto a plate of sterile PDA and were incubated at 28 ± 2°C for 5 days. Discrete pure fungal colonies were stocked on PDA slant, incubated at 28 ± 2°C for 5 days and stored in the refrigerator.

Characterization and identification of fungal isolates

The fungal isolates from the spoilt pawpaw and cucumber fruit samples were characterized and identified using their colonial growth pattern, conidial morphology and pigmentation. Few drops of lacto-phenol cotton blue were placed at the centre of clean, scratch- free microscope slides. A small portion of mycelium of each fungal isolate was picked using a sterile mounting needle and placed in cotton blue-in-lactophenol on a slide; emulsified and cover slip was gently placed at the centre of the slide. Then, the slides were viewed under a light microscope with × 10 and × 40 objective lenses and structure of the mycelium, spore structure and fruiting bodies were appropriately identified (Barnnet and Hunter 1977).

Sources of medicinal plants

Fresh leaves of *Aspilia africana* (Pers.) C. D. Adams were obtained from their natural habitats in Uyo, Akwa Ibom State. The leaves of *A. africana* were transported in zip lock bags and were authenticated by a taxonomist (Prof. Margaret E. Bassey) at the Department of Botany and Ecological Studies, University of Uyo. Thereafter, the plant leaves were taken to Pharmacognosy and Natural Medicine Laboratory, University of Uyo for processing. The *A. africana* leaves were thoroughly washed thrice under running water and rinsed with dH_2O to remove extraneous matters (Onoruvwe and Olorunfemi, 1998; Akinjogunla *et al*., 2011, 2012). The plant leaves were chopped into small pieces, air- dried for 2 weeks under shade at 28 ± 2°C, pulverized using mortar and pestle, and were stored in air-tight polyethylene bag before extraction.

Preparation of the extracts

Aqueous extract

The pulverized leaves of *A. africana* were weighed using a Digital Electronic Laboratory Weighing Balance Scale. The aqueous leaf extract of *A. africana* (ALEAA) was prepared by soaking 1 kg of the pulverized leaves into 2 L of sterile dH_2O for 24 h with occasional shaking at room temperature (26-28°C). Subsequently, Whatman No. 1 filter paper was used to filter the aqueous extract, and the filtrate was dried on a BIOBASE steam water bath (India) at 50°C for 48 h. The dried extract was weighed, preserved in stoppered sample vials and stored in a refrigerator at 4°C (Okoro *et al*., 2012; Akinjogunla and Oluyege, 2016).

Ethanol extract

The pulverized leaves of *A. africana* were weighed using a Digital Electronic Weighing Balance Scale. The ethanol leaf extract of *A. africana* (ELEAA) was prepared by soaking 1 kg of the pulverized leaves into 2 L of ethanol (100%) for 72 h with intermittent shaking. Then, the ethanol extract was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a Buchi rotary evaporator (Flawil, Switzerland) at 50°C for 24 h. The dried extract was weighed, preserved in stoppered sample vials and stored in a refrigerator at 4°C. The graded concentrations of 10, 20, 40 and 80 (mg/mL) of the extracts were prepared using 10% DMSO, shaken vigorously to obtain a homogenous mixture (Akinjogunla *et al.,* 2021; Alozie *et al*., 2023)

Quantitative estimation of secondary metabolites of *A. africana*

The quantitative phytochemical constituents of the leaf extracts of *A. africana* (ALEAA / ELEAA) was determined using the standard procedures. The estimation was done in triplicates and the results expressed as mean and standard deviation.

Estimation of alkaloids

Alkaloids content of the extracts (ALEAA / ELEAA) was determined using the method described by Krishnaiah *et al*. (2009). Five grams (5 g) of the extracts was put into a beaker and 200 mL of 10% CH_3CO_2H in C_2H_5OH was added. This mixture was covered, allowed to stand for 4 h and was filtered. The extract was concentrated in a water bath till it reached twenty-five percent of the original volume. Concentrated NH_4OH was added in drops to the extract till the precipitation was complete. Then, the entire solution was allowed to settle, and the precipitate was collected and washed with dilute NH_4OH and was filtered. The residue obtained was the alkaloids and was dried and appropriately weighed. The percentage alkaloids in the extracts was calculated using Equation 1.

$$\text{Percentage alkaloids} = \frac{\text{FWS}}{\text{WS}} \times 100 \quad (1)$$

Where; FWS: Final weight of sample (g); WS: Weight of sample taken (g).

Estimation of flavonoids

The method described by Boham and Kocipai (1994) was used to determine the flavonoids content of the extracts (ALEAA / ELEAA). Ten grams (10 g) of the extracts samples was extracted repeatedly with 100 mL of 80% aqueous CH_3OH at room temp. The mixture was filtered using Whatman No. 42 filter paper A) and the filtrate was transferred into a beaker and evaporated to dryness over a water bath and thereafter was weighed. The percentage flavonoids in the extracts was calculated using Equation 2.

$$\text{Percentage alkaloids} = \frac{\text{WR}}{\text{WS}} \times 100 \quad (2)$$

Where; WR: Weight of residue (g); WS: Weight of sample taken

Estimation of saponins

Saponins content of the extracts (ALEAA / ELEAA) was determined

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using the method described by Ejikeme *et al*. (2014). Twenty gram (20 g) of the extracts was put into a conical flask and 100 mL of 20 % C_2H_5OH was added. This mixture was heated in a water bath with continuous stirring at about 55°C for 2 h. The mixture was filtered, the extracted residue was added to 100 mL of 20 % C_2H_5OH and the combined extracts were reduced to 40 mL over water bath at about 90 °C. The concentrate was then transferred into a separating funnel and 20 mL $C_4H_{10}O$ was added to the extract and shaken vigorously. The aqueous layer was recovered, the $(C_2H_5)_2O$ layer was discarded and the purification process was repeated. Then, 60 mL of n-C_4H_9OH was added and the combined n-C_4H_9OH was washed twice with 10 mL of 5% NaCl. The residual mixture was heated in a water bath. Then, after evaporation, the samples were dried to a constant weight in the oven. The percentage saponins in the extracts was calculated using Equation 2 above.

Estimation of tannins

The method described by Krishnaiah *et al*. (2009) was used to determine the tannins content of the extracts (ALEAA / ELEAA). Four grams (4g) of the extracts was weighed into 40 mL plastic bottle. Four millilitre dH_2O was shaken for 60 min and was filtered into a 50 mL volumetric flask. Then, 4 mL of the filtrate was mixed with 3 mL of 0.1M $FeCl_3$ in 0.1N HCl and 0.008 M $K_4[Fe(CN)_6]\cdot 3H_2O$ in a test tube. The absorbance was measured at a wavelengths of 120 nm within 10 min in a spectrophotometer. Also, a blank sample was prepared, the colour was developed, and was measured at a wavelengths of 120 nm within 10 min. A standard was prepared with tannin acid to obtain 100 ppm and measured.

Estimation of terpenoids

Terpenoids content of the extracts (ALEAA / ELEAA) was determined using the method described by Krishnaiah *et al.* (2009). Ten gram (10 g) of the extracts was put into a conical flask and 90 mL of 95 % C_2H_5OH was added. The mixture was filtered using Whatman No. 42 filter paper and the filtrate was mixed with 10 mL petroleum ether at 60 °C and was subsequently filtrated using separating funnel. The filtrate was transferred into a beaker and evaporated to dryness over a water bath (65°C) and thereafter was weighed. The percentage terpenoids in the extracts was calculated by using Equation 3.

Percentage (%) terpenoids: $\frac{Wi-Wf}{Wi} \times 100$ (3)

Where; Wi: Weight of dried plant extracts (g); Wf: Weight of extracts after drying

Estimation of cardiac glycoside

Cardiac Glycoside content of the extracts (ALEAA / ELEAA) was determined using the method described by Ejikeme *et al.* (2014). One gram (1g) fine powder of *A. africana* leaves was put into a test tube and 10 mL of 70 % C_2H_5OH was added. The mixture was filtered using Whatman No. 42 filter paper, the filtrate was treated with 5 mL of dH_2O, followed by addition of 1 mL of 12.5% Pb $(C_2H_3O_2)2$ and 2 mL of 4.77% Na_2HPO_4 solution to remove excess phosphorus ions. The residual solution was filtered with filter paper and the filtrate was evaporated to dryness in a water bath. Then, after evaporation, the samples were dried to a constant weight in the oven. The percentage cardiac glycoside in the extracts was calculated using Equation 2 above.

Primary metabolites of *A. africana*

Estimation of proteins

The quantitative estimation of protein in the extracts (ALEAA / ELEAA) was determined using the procedures described by Narasimhan and Sathiyavani (2014). Two grams (2g) of extracts was put into a test tube and 5 mL of freshly prepared alkaline solution (50 mL of 2% Na_2CO_3 in 0.1 N NaOH and 1 mL of 0.5% CuSO4. $5H_2O$ in 1% $KNaC_4H_4O_6\cdot 4H2O$) was added and incubated at room temp for 10 min. Then, 0.5 mL Folin-Ciocalteau reagent was added, properly mixed and kept in dark for 30 mins at room temp until the blue colour developed and absorbance was measured at 660 nm in UV spectrophotometer. The amount of protein present in the extracts was calculated using the standard graph.

Estimation of carbohydrate

The quantitative estimation of carbohydrate in the extracts (ALEAA / ELEAA) was determined using the anthrone method (Nayaka and Londonkar, 2019). One gram (1g) of extracts was well mixed with 4 mL of anthrone reagent (200 mg of anthrone in 100 mL of ice cold 95% H_2SO_4) in a test tube. Then, the mixture was incubated in boiling water bath for 8 min and the absorbance was read at 630 nm against a reagent blank. The estimation was done in triplicates and the results expressed as mg/g of sample.

Estimation of lipids

The quantitative estimation of lipids in the extracts (ALEAA / ELEAA) was determined using the method described by Nayaka and Londonkar (2019). Ten (10 g) of extracts was loaded into the thimble and placed inside the Soxhlet extractor using 150 mL petroleum ether and was left for 16 h. The obtained extract was concentrated and evaporated to dryness. The weight of extract obtained was the lipid and it was expressed as mg/g dry weight sample.

***In vitro* antifungal susceptibility testing of the extracts**

The ALEAA / ELEAA were assayed for antifungal activities *in vitro* by disc diffusion method (Akinjogunla and Eghafona, 2012, Akinjogunla *et al*., 2021). 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 of 10, 20, 40 and 80 (mg/mL) and these discs were placed on PDA plates which had been previously inoculated with the mycelium of fungal isolates. DMSO was used as negative control. The plates were incubated at room temp for 5 days. The experiments were performed in triplicates. The culture plates were observed for the zone of inhibition around the discs which displayed the antifungal activity of the extracts after the incubation and results were expressed as mean and standard deviation (x ± S.D).

Statistical analysis

The Statistical Package for the Social Science (version 22.0) was used for analysis. Pearson's correlation between primary and secondary metabolites were determined. Regression analysis was used to assess the relationship between concentrations of extracts and antifungal activity vis-a vis the diameters of inhibitory zones and isolate at 95% levels of significance.

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RESULTS

Based on the colonial and morphological characteristics (shape, type of soma, nature of hyphae, pseudo-mycelium and asexual reproductive spore), the fungal isolates obtained from the spoilt *C. sativus* and *C. papaya* fruits were *A. niger, P. notatum, A. flavus, Mucor* spp, *R. stolonifer,* and *Fusarium* spp. The percentage occurrences of *A. niger, P. notatum, A. flavus, Mucor* spp, *R. stolonifer* and *Fusarium* spp associated with spoilt *C. sativus* fruits are shown in Fig 1. In spoilt *C. sativus* fruits, *A. niger* had the highest percentage occurrence (40%), followed by *Fusarium* spp with 30%, while *Mucor* spp had the lowest percentage occurrence (10%). In spoilt *C. papaya* fruits, the percentage occurrence of *A. niger* and *P. notatum* was 30% each; *A. flavus* and *Mucor* spp was 20% each, while *Fusarium* spp was 10% (Fig 2).

The quantitative estimations of primary and secondary metabolites in leaf extracts of *A. africana* were determined and are presented in Tables 1. The ALEAA had mean protein, carbohydrate and lipid contents of 15.36 ± 0.32%, 60.97 ± 1.14% and 6.66 ± 0.04%, respectively. In the ELEAA, the mean carbohydrate content was the highest (61.23 ± 0.42%), followed by protein content (16.19 ± 0.06%) and lipid content (6.57 ± 0.04%). The analysis revealed that the mean values of secondary metabolites (flavonoids and saponins of 2.28 ± 0.04% and 4.32 ± 0.01% w/w, respectively) in the ALEAA were slightly higher than the values of secondary metabolites (flavonoids and saponins of 2.09 ± 0.01% and 4.15 ± 0.03% w/w, respectively) in the ELEAA. The mean alkaloids and terpenoids contents of the ALEAA and ELEAA ranged from 8.12 ± 0.12 % to 8.27 ± 0.02 % and 0.30 ± 0.01% to 0.37 ± 0.01%, respectively. The mean cardiac glycosides and tannins contents in ALEAA was 1.21 ± 0.01% and 2.43 ± 0.02% respectively, compared to 2.19 ± 0.02% and 3.23 ± 0.12% obtained for cardiac glycosides and tannins contents in the ELEAA (Table 1).

Table 2 shows the Pearson's correlations between primary and secondary metabolites of the ALEAA. Carbohydrate (CHO) showed a very strong positive correlation with saponins and a strong negative correlation with terpenoids, with a correlation coefficient (r) of 0.9262 and - 0.7896, respectively ($p \leq 0.05$). Alkaloids was observed to have a weak positive correlation with protein (r = 0.2028) and CHO (r = 0.421), and a negative correlation with lipid (r = -0.6556) at $p < 0.05$. Similarly, a high positive relationship was exhibited between cardiac glycosides and lipid (r = 0.9286) at $p < 0.05$. The Pearson's correlations between primary and secondary metabolites of ELEAA are shown in Table 3. The saponins exhibited a very strong positive relationship with protein (r = 0.9286) and CHO (r = 0.9959) at the 0.05 level. Flavonoids, tannins and terpenoids showed a strong positive relationship with CHO (r = 0.9707); CGS exhibited a moderate positive relationship with protein (r^2 = 0.5), while a high negative relationship (r = -0.9999) at the 0.05 level was shown by CGS and Lipid (Table 3).
The varied concentrations (10, 20, 40 and 80 mg/mL) of ALEAA and ELEAA demonstrated antifungal activity *A. niger, P. notatum, A. flavus, Mucor* spp, *R. stolonifer* and *Fusarium* spp isolated from spoilt *C. sativus* and *C. papaya* fruits (Tables 4 and 5). The ELEAA displayed antifungal activity against A. *niger* with mean zone of inhibitions (ZIs) ranging from 10.1 ± 0.1 mm to 18.8 ± 0.3 mm. The means ZIs obtained when ELEAA was tested against P. *notatum* ranged between 11.6 ± 0.1 and 17.1 ± 1.1 mm. The highest mean ZI (x ± S.D) of 13.5 ± 0.5 mm and lowest mean ZI (x ± S.D) of 9.3 ± 0.1 mm were obtained when ELEAA was tested against *Fusarium* spp. At a concentration of 10 and 20 (mg/mL), the ALEAA did not exhibit antifungal activity against *Mucor* spp MSCP-08 and MSCS-14 (Table 4). The ALEAA displayed antifungal activity against *A. niger* with mean ZI ranging from 9.4 ± 0.4 mm as obtained from the plate containing *A. niger* ANMI-09 to 16.0 ± 1.0 mm as obtained from the plate containing *A. niger* ANCP-12. The highest mean ZI (x ± S.D) of 15.9 ± 1.0 mm and the lowest mean ZI (x ± S.D) of 9.9 ± 0.1 mm were obtained when ALEAA was tested against *P. notatum*. At a concentration of 10 mg/mL, the ALEAA did not exhibit antifungal activity against *Fusarium* spp FSCS-15, *R. stolonifer* RSCA-05, *Mucor* spp MSCP-08 and MSCS-14 (Table 6). At a concentration of 10 mg/mL, the ALEAA did not exhibit antifungal activity against *Fusarium* spp FSCS-15 (Table 4).

The regression coefficients (R) between 10, 20, 40 and 80 (mg/mL) concentration of extracts (ALEAA / ELEAA) and diameters of IZs as exhibited by *A. niger, P. notatum* and *Fusarium* spp from spoilt cucumber and pawpaw are presented in Table 6. The R values of ELEAA and diameters of IZs as exhibited by the fungal isolates ranged from 0.6324 to 0.9936, while the R values of ALEAA and diameters of IZs as exhibited by the fungal isolates ranged from 0.6495 to 0.9876 (Table 6).

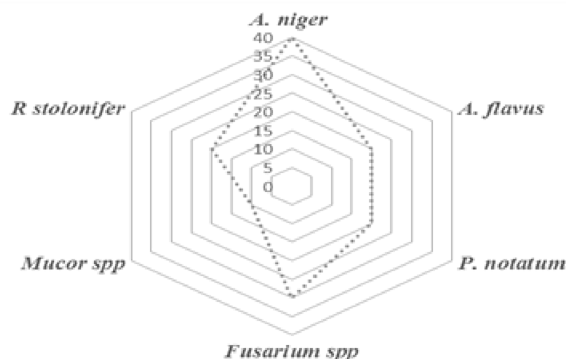

Fig 1: Percentage of Occurrences of Fungal Isolates in Spoilt *C. sativus* Fruits

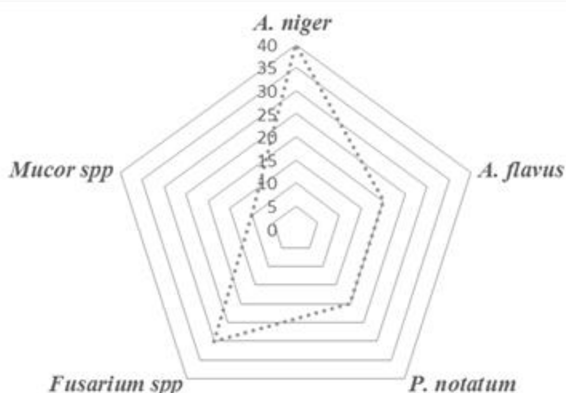

Fig 2: Percentage of Occurrences of Fungal Isolates in Spoilt *C. papaya* Fruits

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Table 1: Quantitative estimation of metabolites of leaf extracts of *A. africana*

Phytochemical Constituents		x ± S.D (%)	
		ALEAA	ELEAA
Primary metabolites	protein	15.36 ± 0.32	16.19 ± 0.06
	carbohydrates	60.97 ± 1.14	61.23 ± 0.42
	lipids	6.66 ± 0.04	6.57 ± 0.04
Secondary metabolites	alkaloids	8.12 ± 0.12	8.27 ± 0.02
	flavonoids	2.28 ± 0.04	2.09 ± 0.01
	saponins	4.32 ± 0.01	4.15 ± 0.03
	tannins	2.43 ± 0.02	3.23 ± 0.12
	terpenoids	0.30 ± 0.01	0.37 ± 0.01
	cardiac glycosides	1.21 ± 0.01	2.19 ± 0.02

Keys: x: mean; S.D: Standard Deviation; ALEAA: Aqueous Leaf Extracts of *A africana;* ELEAA: Ethanol Aqueous Leaf Extracts of *A. africana*

Table 2: Correlation matrix between primary and secondary metabolites of ALEAA

	Protein	CHO	Lipid	Alkaloids	Flavonoids	Saponins	Tannins	Terpenoids	CGS
Protein	1								
CHO	0.9736	1							
Lipid	-0.8724	-0.9609	1						
Alkaloids	0.2028	0.4211	0.6556	1					
Flavonoids	0.9992	0.9634	-0.8515	0.1623	1				
Saponins	0.9878	0.9262	-0.7857	0.0481	0.9934	1			
Tannins	0.8825	0.7518	-0.5399	-0.2815	0.9011	0.9449	1		
Terpenoids	-0.9333	-0.9906	0.9897	-0.5411	-0.9177	-0.8660	-0.6546	1	
CGS	-0.6286	-0.7896	0.9286	-0.8891	-0.5961	-0.5	-0.1889	0.866	1

Keys: CHO: Carbohydrate; CGS: Cardiac Glycosides; ELEAA: Ethanol Leaf Extracts of *A. africana.*

Table 3: Correlation matrix between primary and secondary metabolites of ELEAA

	Protein	CHO	Lipid	Alkaloids	Flavonoids	Saponins	Tannins	Terpenoids	CGS
Protein	1								
CHO	0.8917	1							
Lipid	-0.5	-0.0524	1						
Alkaloids	0.6546	0.2402	-0.9819	1					
Flavonoids	0.756	0.9707	0.1889	0.0615	1				
Saponins	0.9286	0.9959	-0.1429	0.3273	0.9449	1			
Tannins	0.7559	0.9707	0.1889	0.0904	0.9998	0.9449	1		
Terpenoids	0.7559	0.9907	0.1889	0.0122	0. 9975	0.9449	0.9999	1	
CGS	0.5	0.0524	-0.9999	0.9827	-0.1889	0.1419	-0.1889	-0.1889	1

Keys: CHO: Carbohydrate; CGS: Cardiac Glycosides; ELEAA: Ethanol Leaf Extracts of *A. Africana*

Table 4: Antifungal activities of ethanol leaf extracts of *A. africana* (ELELA)

Fungal Isolates	Code of Isolate	Zone of inhibition (x ± S.D)				
		10 mgmL^{-1}	20 mgmL^{-1}	40 mgmL^{-1}	80 mgmL^{-1}	10% DMSO
A. niger	ANCS-01	12.1 ± 0.2^a	15.0 ± 1.0^b	15.5 ± 0.5^b	17.2 ± 1.2^b	NZ
	ANCP-12	14.2 ± 0.2^a	16.3 ± 1.0^a	18.1 ± 1.1^b	18.8 ± 0.3^b	NZ
	ANMI-09	10.1 ± 0.1^a	11.6 ± 0.2^a	13.0 ± 0.0^b	14.2 ± 0.2^b	NZ
P. notatum	PNCP-03	13.0 ± 0.5^a	14.8 ± 0.3^a	16.6 ± 0.5^b	17.1 ± 1.1^b	NZ
	PNCA-10	11.6 ± 0.1^a	12.3 ± 0.3^a	14.0 ± 0.0^b	14.4 ± 0.3^b	NZ
Fusarium spp	FSCS-15	9.3 ± 0.1^a	10.1 ± 0.1^a	11.4 ± 0.3^a	13.5 ± 0.5^b	NZ
	FSMI-19	10.5 ± 0.5^a	10.6 ± 0.5^a	12.0 ± 1.0^a	12.9 ± 0.2^a	NZ
A. flavus	AFCS-11	12.6 ± 0.2^a	13.4 ± 0.5^a	15.2 ± 1.1^b	15.7 ± 0.5^b	NZ
	AFCA-06	10.2 ± 0.2^a	11.5 ± 0.5^a	13.7 ± 0.2^b	15.0 ± 1.0^b	NZ
R. stolonifer	RSCA-05	9.8 ± 0.2^a	11.1 ± 0.1^a	13.2 ± 0.2^b	13.9 ± 0.1^b	NZ
Mucor spp	MSCP-08	N Z	N Z	9.9 ± 0.1^a	11.2 ± 0.2^a	NZ
	MSCS-14	N Z	N Z	10.5 ± 0.0^a	13.4 ± 0.1^a	NZ

Keys: x: mean; SD: Standard Deviation; NZ: No zone of inhibition; each inhibitory zone included 6 mm diameter of the disc; mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test ($P < 0.05$).

Table 5: Antifungal activities of aqueous leaf extracts of *A. africana* (ALELA)

Fungal Isolates	Code of Isolate	Zone of inhibition (x ± S.D)				
		10 mgmL^{-1}	20 mgmL^{-1}	40 mgmL^{-1}	80 mgmL^{-1}	10% DMSO
A. niger	ANCS-01	11.5 ± 0.1^a	12.7 ± 0.2^a	14.2 ± 1.2^b	15.7 ± 1.1^b	NZ
	ANCP-12	12.6 ± 0.3^a	14.9 ± 1.5^a	15.4 ± 1.0^b	16.0 ± 1.0^b	NZ
	ANMI-09	9.4 ± 0.4^a	9.9 ± 0.1^a	10.5 ± 0.5^a	12.7 ± 0.5^a	NZ
P. notatum	PNCP-03	12.5 ± 0.5^a	13.3 ± 0.2^a	15.0 ± 0.5^b	15.9 ± 1.0^b	NZ
	PNCA-10	9.9 ± 0.1^a	10.5 ± 0.1^a	12.8 ± 0.2^a	13.1 ± 0.1^a	NZ
Fusarium spp	FSCS-15	N Z	8.6 ± 0.2^a	10.0 ± 0.0^a	11.8 ± 0.2^a	NZ
	FSMI-19	9.8 ± 0.2^a	11.3 ± 0.1^a	12.4 ± 0.3^a	12.8 ± 0.2^a	NZ
A. flavus	AFCS-11	11.2 ± 0.2^a	12.7 ± 0.2^a	14.0 ± 1.0^b	14.3 ± 0.3^b	NZ
	AFCA-06	10.0 ± 0.0^a	10.8 ± 0.2^a	11.4 ± 0.1^a	13.7 ± 0.2^b	NZ
R. stolonifer	RSCA-05	N Z	8.6 ± 0.1^a	10.2 ± 0.2^a	12.2 ± 0.2^a	NZ
Mucor spp	MSCP-08	N Z	N Z	8.1 ± 0.0^a	9.5 ± 0.0^a	NZ
	MSCS-14	N Z	N Z	8.9 ± 0.1^a	11.1 ± 1.1^a	NZ

Keys: x: mean; SD: Standard Deviation; NZ: No zone of inhibition; each inhibitory zone included 6 mm diameter of the disc; mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test ($P < 0.05$).

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)	
		ELEAA	ALEAA
A. niger	ANCS-01	0.7851	0.9414
	ANCP-12	0.6324	0.6495
	ANMI-09	0.8912	0.9876
P. notatum	PNCP-03	0.7667	0.9000
	PNCA-10	0.8207	0.7871
Fusarium spp	FSCS-15	0.9936	0.5985
	FSMI-19	0.9358	0.7468
A. flavus	AFCS-11	0.8328	0.7325
	AFCA-06	0.9008	0.9872
R. stolonifer	RSCA-05	0.8322	0.6220
Mucor spp	MSCP-08	0.7777	0.7974
	MSCS-14	0.8398	0.8288

Keys: ALEAA: Aqueous Leaf Extracts of *A. africana;* ELEAA: Ethanol Aqueous Leaf Extracts of *A. Africana*.

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DISCUSSION

Fruits are frequently displayed in the baskets and / or on benches for customers to purchase. The display of these fruits could cause microbial infection (Chukwuka *et al*., 2010), and colonization of fruits by the invading microorganisms is a critical stage in the microbial spoilage of fruits (Ijato *et al*., 2021). The fungal isolates from both spoilt *C. sativus* and *C. papaya* fruits in this study were *A. niger, P. notatum, Fusarium* spp, *A. flavus* and *Mucor* spp. Isolation of fungi in genera Aspergillus in our study substantiated the findings of Baiyewu *et al*. (2007) and Chukwuka *et al.* (2010) who isolated *Aspergillus* spp from *C. papaya* fruits in South-western Nigeria. The occurrence of *Fusarium* spp in *C. sativus* agrees with Majdah (2015) that *Fusarium* spp was often associated with spoilage of *C. sativus* fruits. Microbial spoilage may be due to pathogens acting on fruits, stems, leaves, flowers, root or other parts of the plants.

Analysis of primary and secondary metabolites contents in ALEAA and ELEAA revealed that mean percentages of the primary metabolites were higher than that of secondary metabolites in the present study and this however, contradicts the findings of Amani *et al*. (2017) who observed that the primary metabolites mean percents were lower than secondary metabolites in plant extracts. The mean alkaloids contents of 8.12 ± 0.12 % in the ALEAA in our study was similar to 8.22 ± 0.40 % reported by Adegbesan (2019) on the quantitative phytochemical screening of aqueous extract of *A. africana* in Abeokuta, Ogun State, Nigeria. This moderate quantity of alkaloid in ALEAA substantiated the previous work of Agbor *et al*. (2012). We obtained a moderately high protein contents in the ALEAA and ELEAA and this was consistent with the values reported by Ojebiyi *et al.* (2013) and Adedeji *et al.* (2015) in Oyo and Osun States, Nigeria. The detection of these secondary metabolites in ALEAA and ELEAA in this study validated the results of Ekaiko *et al*. (2015) who reported flavonoids, saponins, tannins and cardiac glycoside as the phytochemical constituents present in *A. africana*. Generally, the mean percentages of four secondary metabolites (alkaloids, tannins, terpenoids and cardiac glycosides) in ELEAA were higher than that of the ALEAA, but the ALEAA was richer in flavonoids and saponins than ELEAA. Thus, polarity level of the solvents must have played a major role in the extraction of secondary metabolites (Ghasemzade *et al*., 2011).

In our study, flavonoids, tannins and terpenoids showed a strong positive relationship with CHO; CGS exhibited a moderate positive relationship with protein at the 0.05 level was shown by CGS and Lipid. The positive correlations observed between the metabolites in this study indicate that changes in primary metabolite contents are associated with changes in the secondary metabolite contents of the extracts.

Our findings on antifungal activities of the ALEAA and ELEAA against fungal isolates from spoilt fruits corroborated the previous reports on the antimicrobial activity of the leaves of *A. africana* by Johnson *et al*. (2016). In this present study, the ELEAA showed more growth inhibitory effects on test fungal isolates as exhibited by wider zones of inhibition than the ALEAA. This may be attributed to variations in the dissolution capacity of the different solvents which in turn affected the degree of phytochemicals extracted (Ijato *et al*., 2021). The diameter zone of inhibition (mm) increased with increased extract concentrations and similarly this has been reported by Woldeyes *et al.* (2012).

The R values of ELEAA and diameters of IZs as exhibited by the fungal isolates ranged from 0.6324 to 0.9936, while the R values of ALEAA and diameters of IZs as exhibited by the fungal isolates ranged from 0.6495 to 0.9876. Our finding on the regression coefficient (R) in the present study is consistent with the results of Ijato *et al*. (2021) on the regression analysis between concentration of extracts and diameters of zones of inhibition as exhibited by fungal isolates.

Conclusion

The present study has unveiled additional detailed findings and information regarding the quantitative phytochemical constituents of aqueous and ethanol leaf extracts of *A. africana* and as well revealed its considerable antifungal activities against pathogenic fungi associated with post-harvest spoilage of *C. papaya* and *C. sativus* fruits. Consequently, the utilization of aqueous and ethanol leaf extracts of *A. africana* as a potential alternative preservative of *C. papaya* and *C. sativus* fruits against spoilage by fungi could be highly considered.

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