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Phytochemical and Antifungal Activity of *Kigelia Africana* and *Artocarpus Communis*

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ABSTRACT

Food spoilage and food losses became an important issue for human beings as regard the food safety and food security, since people started producing and storing food products. This study aims at investigating the antifungal activity of the leaves of *Kigelia Africana and Artocarpus communis.* Twenty grams of each plant was weighed and then soaked in 100 ml of distilled water, hot water and also organic solvent and they were shaken at intervals and left for 24 hours. The extracts were sieved using cheese cloth then centrifuged, and afterward filtered using Whatman no 1 filter paper. These extract solutions (100%) were diluted with water to give 75% concentration of the extracts while distilled water served as control. After extraction, phytochemical screening was carried out on the extracts. Isolation of test organism from spoilt yoghurt and bread was carried out using pour plate and serial dilution method. Fungi isolates were identified based on cultural characteristics, morphological examination and also using fungi atlas. Test organisms ; *Rhizopus* sp, *Candida* sp and *Fusarium* sp were subjected to antibacterial effect of varying concentration of these extracts using agar well diffusion method. The data obtained were analyzed using ANOVA. The screening of the methanol and aqueous extract of *K. Africana and A. communis* indicated the presence of glycosides, flavonoids, phenol, saponins, quinone, steroid and terpenoids. There was antifungal activity of both methanol and aqueous extract of *K. Africana and A. communis.* The aqueous extract exhibited antifungal activity against the test organism at varying concentrations with zone of inhibition values ranging from 26.00±2.00 to 12.00±3.00 for *K. africana* while the range of inhibition zone for *A. communis* range from 27.00±3.00 to 13.50±1.50. The leaf extract of *K. Africana and A. communis* have broad spectrum of antimicrobial activity. The extract of *K. Africana and A. communis* showed antifungal activity and hence might be useful in the industry in replacing synthetic preservative.

Key words: *Kigelia africana, Artocarpus communis*, extracts, phytochemical, fungi

1.0 INTRODUCTION

Food spoilage and food losses are important issues for human beings with regards to food safety and food security, since people started producing and storing food products. Up to one third of all food is ruined or wasted before consumption, which represents about 1.3 billion tons per year (Rezaei and Liu, 2017). These losses result to one or more problems occurring in the supply chain, beginning from the agricultural production down to the consumer level. Regarding food spoilage, a food product can be physically, chemically, or microbiologically spoiled. The main agents responsible for microbial spoilage include parasites, bacteria, and/or fungi.

There is a considerable concern among consumers regarding the risk of using synthetic additives for human health, that led to decrease the use of these chemicals in food preservation (Caleja *et al*., 2016).

Kigelia africana belong to the family Bignoniaceae and Native of West Tropical Africa. *Artocarpus communis*is a tree of the mulberry family (Moraceae) and its large fruits that are a staple food of the South Pacific and other tropical areas. Extract of *K. africana* and *A. communis* have been found to have important phytochemical that can be used a natural preservative.

Consumers in recent years have high preference for natural preservatives to chemical-based preservatives due to their adverse health effects. This natural preservative are alternatives to chemical preservatives so as to prolong the shelf life and safety of food (Arora *et al.,* 2013 ; Tavasalkar *et al.,* 2012). The preservation of food crops is necessary to prevent and reduce spoilage caused by microorganisms. It also helps to increase the shelf life of food crops and meet the needs of the demands of consumers for safe and natural products without chemical preservatives.

Therefore, the objective of this study was to determine the phytochemicals in the extracts of *Kigelia africana and Artocarpus communis* and examine the effects of the extracts on the growth of fungi in spoilt food.

2.0 MATERIALS AND METHODS

2.1. COLLECTION OF PLANTS AND EXTRACTION.

Extraction procedures was carried out according to standard methods. *Kigelia africana, and Artocarpus communis* were collected and identified by a Botanist in the Department of Biological Sciences, Anchor University, Lagos. The leaves were thoroughly washed and placed in the shade for drying within the laboratory at room temperature and the leaves were grinded into powder using blender

Twenty grams of powdered sample was weighed and poured into a conical flask and then 100 mL of distilled water was measured using a measuring cylinder and then poured into the 500 mL conical flask containing the powdered sample to obtain distilled water extract. Hot water extract was prepared by boiling the distilled water extract to obtain hot water extract of the different concentrations. Thereafter soaked for 24 hours and shaken at intervals using the mechanical shaker and after then it was filtered using muclin cloth. The supernatant was centrifuged using a centrifuge at 10,000 rpm for 5 minutes for separating the extra debris from the solution. It was refiltered using a filter paper to get a more purified extract. From that solution various concentrations of aqueous extracts were prepared which includes 100%,75%,50% and 25% by the way of dilution and then stored. Twenty grams of powdered sample was weighed using electronic scale and poured into a conical flask and then 100 mL of methanol was measured using a measuring cylinder and then poured into the 500 mL conical flask containing the powdered sample. Thereafter, soaked for 24 hours and shaken at intervals using the mechanical shaker and after then it was filtered using muclin cloth. The supernatant was centrifuged using a centrifuge at 10,000 rpm for 5 minutes for separating the extra debris from the solution. It was refiltered using a filter paper to get a more purified extract. From that solution various concentrations of methanolic extract were prepared which includes 100%,75%,50% and 25% by the way of dilution and the stored in the refrigerator

2.2 PHYTOCHEMICAL ANALYSIS

Phytochemical screening for alkaloids, phenols, flavonoids, saponins, terpenoids and glycosides, steroids, quinones and anthraquinones, sulphuric acid was carried according to Sofowora (1982).

2.3 ISOLATION AND IDENTIFICATION OF TEST ORGANISMS

The Fungi were isolated from spoiled food samples (yoghurt and bread) by using the pour plate method. Observable changes showing on the food samples were inoculated using sterile inoculating needle and suspended in 5 mL of Tryptic soy broth.

1 mL from each dilution was inoculated into sterile labeled Petri dishes. Potato dextrose agar plates were inoculated with 1 ml of each diluent and incubated at 25ºC for 3-5 days for fungal growth and pure fungal isolates were stored on PDA slants for further use. Fungi isolates were identified based on cultural characteristics and morphological examination (Kidd *et al.*, 2016).

2.4 ANTIFUNGAL ASSAY

Agar well diffusion method was used to screen the antibacterial and antifungal activities of different solvent extracts as displayed by Daoud *et al*., (2015). One mL of fresh fungi culture will be pipetted in the center of sterile Petri dish. Mueller Hinton Agar (MHA) was poured into the Petri dish containing the inoculum and mixed well. Upon solidification, wells were made using a sterile corkborer (6 mm in diameter) into agar plates containing inoculums. Different concentrations of the extracts (100 µl of each extract) (100 mg/ml, 75 mg/ml) were introduced into the different bored wells (6 mm corkborer) in each plate. The plates were incubated at 25°C for 18 hours. Antimicrobial activity was detected by measuring the zone of inhibition (including the wells diameter) that appeared after the incubation period. A control using distilled water was also prepared and introduced into the well.

2.5 STATISTICAL ANALYSIS

The data obtained were analysed by factorial Analysis of Variance (ANOVA) to determine significant ($P< 0.05$) effects. The significant differences between means were determined using Duncan's Multiple Range Test DMRT. The result of the study is presented as Mean ± standard error of the trials.

3. RESULTS

3.1. Phytochemical Screening of *K. africana* and *A. communis* Aqueous and methanolic extracts.

From the analysis carried out on extracts of *K. africana*, all phytochemicals were found in the hot water and distilled water extract except alkaloid. Analysis indicated the presence of phenols and saponins in the methanol extract (table 1). From the analysis carried out on the hot water extract of *A. communis*, glycosides, flavonoid and saponins were absent while phenols, terpenoids, steroid and quinone were present. Analysis indicated the presence of flavonoid, saponins, phenols, terpenoids, steroid and quinone in the distilled water extract while glycosides were absent. The analysis on methanolic extract indicated absence phytochemical. Alkaloid was totally absent for hot water, distilled water and methanol extract (table 2).

Table 1: Phytochemical screening of *K. africana* extract

Phytochemicals	Hot water	Distilled water	Methanol
Alkaloid	_	_	_
Glycosides	+	+	_
Flavonoids	+	+	_
Saponins	+	+	+
Phenols	+	+	+
Terpenoids	+	+	_
Steroids	+	+	_
Quinones	+	+	_

\+ indicates the presence of the phytochemical compound
_ indicates the absence of the phytochemical compound

Table 2: phytochemical screening of *A. communis* extract

Phytochemicals	Hot water	Distilled water	Methanol
Alkaloid	_	_	_
Glycosides	_	_	-
Flavonoids	_	+	_
Saponins	_	+	_
Phenols	+	+	_
Terpenoids	+	+	_
Steroids	+	+	_
Quinones	+	+	_

\+ indicates the presence of the phytochemical compound
_ indicates the absence of the phytochemical compound

3.2 ANTIFUNGAL ASSAY

Table 3 shows the antifungi activity of the aqueous extract and methanolic extract of the leaf of *Kigelia africana* against *Rhizopus* sp, *Candida* and *Fusarium* sp. The hot water extract was more effective against all the microorganisms compared to the distilled water extract. From the zone of inhibition, the methanol extract showed no activity against *Candida* sp and *Fusarium* sp. The highest activity zone of inhibition of 26.00 ± 2.00 mm of hot water extract was observed against *Rhizopus* sp at 100 % concentration. All zones of inhibitions for all organisms were significantly different at different concentrations.

The antifungi activity of the aqueous extract and methanol extract of the leaf of *Artocarpus communis* against *Rhizopus* sp, *Candida* and *Fusarium* sp is shown in Table 4. The hot water extract was more effective against all the microorganisms compared to the distilled water and methol extract. The highest activity zone of inhibition of 27.00 ± 3.00 was observed in hot water extract against *Rhizopus* sp at 75 % concentration.

Table 4: Antifungal activity of *Kigelia Africana* against *Rhizopus* sp, *Candida* sp, *Fusarium* sp

		***Rhizopus* sp**	***Candida* sp**	***Fusarium* sp**
***K. africana* extract**	**Extract conc**	**Zone of inhibition**	**Zone of inhibition**	**Zone of inhibition**
Distilled water	100 %	14.00 ± 4.00	19.00 ± 1.00	18.00 ± 6.00
	75 %	17.00 ± 2.00	16.50 ± 0.50	12.00 ± 3.00
Hot water	100%	26.00 ± 2.00	22.00 ± 2.00	19.50 ± 0.50
	75%	22.00 ± 2.00	17.50 ± 2.50	17.00 ± 1.00
Methanol	100%	17.00 ± 8.00	-	-
	75%	13.00 ± 4.00	-	-

Table 5: Antifungal activity of *Artocarpus comminis* against *Rhizopus* sp, *Candida* sp, *Fusarium* sp

		***Rhizopus* sp**	***Candida* sp**	***Fusarium* sp**
***A. communis* extract**	**Extract conc**	**Zone of inhibition**	**Zone of inhibition**	**Zone of inhibition**
Distilled water	100 %	22.50 ± 2.50	14.50 ± 1.50	21.50 ± 2.50
	75 %	19.50 ± 4.50	13.50 ± 1.50	15.00 ± 5.00
Hot water	100%	20.00 ± 0.00	17.50 ± 2.50	22.00 ± 2.00
	75%	27.00 ± 3.00	13.50 ± 1.50	14.50 ± 2.50
Methanol	100%	17.00 ± 8.00	-	-
	75%	20.00 ± 11.50	-	-

4. DISCUSSION

Fungal contamination of food is a very serious problem in tropical warm regions of the world. Contamination by storage fungi and their mycotoxins is of great concern in food industry. A recent trend in food processing is to avoid the use of chemical preservatives. Thus, natural antimicrobial alternatives are required. Several researchers have reported that plants contain bioactive substances (Kilani, 2006 ; Babu *et al*., 2007 ; Maswada and Elzaawely, 2013). The results of the present study corroborate the reports of previous workers as there was presence of glycosides, phenols, alkaloids, terpenoids, flavonoids and saponins, steroid, anthraquinone in the plant extracts used for this study.

Several researchers investigated the efficiency of plant extracts and their effective compounds as antimicrobial agents to control growth of food borne and spoilage fungi.

In this study *Rhizopus* sp, were isolated from spoilt bread which is in agreement with Daoud *et al., (*2015) who isolated *Rhizopus* sp from spoilt bread. This study showed that the plant extract had significant antimicrobial activity against the isolated organisms.

Also, from this study *Candida sp, and Fusarum* sp was isolated from yoghurt which is in agreement with (Fatima *et al.*, 2009) who worked on the antifungal effects of *G. glabra* extract on *Candida* sp and *Fusarum* sp. *Kigelia africana* and *Artocarpus communis* showed significant antifungal efficacy against these organisms. Also from this study, the fungi were susceptible to the extracts and the different extracts varied in their effectiveness in inhibiting fungi growth. Some studies have

reported antifungal activity of essential oil, ethanolic and aqueous extracts of *S. molle* (Schmourlo *et al*., 2005). The results of this current research are in agreement with other finding (Arif *et al*. 2009 Khan and Yadav, 2011; Sangvikar *et al*. 2012)

Comparison of the growth inhibition of the extracts shows a dependent effect on extract concentrations. In general, the antifungal activity of 75% extract is weaker compared to 100% extracts. These results revealed that antifungal activity of the extracts was enhanced by increasing the concentration of the extracts, in effect, the inhibition activity of the extracts was concentration dependent. This finding is in agreement with the report of Anchana and Jennifer (2014), who also observed that higher concentrations of antimicrobial substances showed more growth inhibition.

Plant extracts are generally a mixture of active and non-active compounds. The antimicrobial activity of plant extracts might not be due to the action of a single active compound, but the synergistic effect of several compounds. Studies have shown that the antimicrobial activity of plants might be due to the presence and synergistic activity of diverse bioactive metabolites (Manilal and Idhayadhulla, 2014).

5.CONCLUSION

The aqueous extracts of *Kigelia africana*, and *Artocarpus communis* exhibited a varying degree of activities against the fungi spoilage organisms. Fungi cause enormous problems in the plant production industry and inadequate proper control can lead to serious problems in food production. The present study suggested that plant extracts which proved to be potentially effective can be used as natural preservatives to preserve food thereby avoiding use of chemical preservatives.

Declaration by Authors

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