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ANTIMICROBIAL ACTIVITY OF METHANOL AND AQUEOUS EXTRACTS OF *DACRYODES EDULIS* AGAINST FOOD BORNE MICROBES

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Competing Interests.
The authors declare no competing interests.

ABSTRACT

Background: 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. **Objective**: This study was designed to examine the antimicrobial effect of extracts from *Dacryodes edulis*. **Methods**: Ten grams of *Dacryodes edulis* was extracted in 100 ml of distilled water, hot water and methanol. 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. The phytochemical screening was carried out to check for the bioactive compounds present in the plant extract. The isolation of the organisms from the spoilt beef and chicken was done using standard method. Thereafter, the organisms were sub-cultured in other to obtain a pure culture. The identified organisms are *Salmonella enterica, Proteus vulgaris, Citrobacter freundii, Trycophyton and Cladosporium.* These organisms were then subjected to antimicrobial activity of the methanol and aqueous extract using the agar well diffusion method. The data obtained were analyzed using ANOVA via SPSS. **Results**: The identified organisms are *Citrobacter freundii*, *Salmonella enterica, Proteus vulgaris, Trycophyton* and *Cladosporium*. The screening of the extracts of *Dacryodes edulis* indicated the presence of phenol, alkaloid, glycoside, steroid, quinon, terpenoid, antraquinon, and flavonoid. The results showed antibacterial and antifungal activity of both methanol and aqueous extracts of *D. edulis.* **Conclusion**: The extracts of *D. edulis* showed antimicrobial activity and activities of the extracts were dose dependent. Hence the extracts might be used in preservation of food. against *Salmonella enterica, Proteus vulgaris, Citrobacter freundii, Trycophyton and Cladosporium.*

Keywords: *Dacryodes edulis,* Crude extracts, Antimicrobial activity, Food-borne microbes.

INTRODUCTION

Food losses have an impact on food security for poor people, on food quality and safety, on economic development and on the environment (Godfray *et al.,* 2010). The exact causes of food losses vary throughout the world and are very much dependent on the specific conditions and local situation in a given country (Rawat, 2015). Irrespective of the level of economic development and maturity of systems in a country, food losses should be kept to a minimum (Chalwe, 2011).

Food losses represent a waste of resources used in production such as land, water, energy and inputs (Kummu *et al.,* 2012). Economically avoidable food losses have a direct and negative impact on the income of both farmers and consumers (Heller and Keoleian, 2015). For poor consumers (food insecure or at-risk households), the priority is clearly to have access to food products that are nutritious, safe and affordable (Holben, 2010). It is important to note that food insecurity is often more a question of access than a supply problem. Food production must increase significantly to meet future global demand (Ziervogel *et al.,* 2005).

An internationally acceptable standard in food quality emphasized that food (processed or raw) should be wholesome and free of contaminants (Salgueiro *et al.,* 2010). Food borne pathogens are the leading causes of illness and death in undeveloped countries, billing approximately 1.8 million people annually (Osunla and Okoh, 2017). Fungi are the major cause of food deterioration and spoilage worldwide,

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ranking second to insects (Pitt and Hockling, 2009). To prevent spoilage is food several physical and chemical preservation techniques are commonly employed. Increasing consumers demand for green food products with high safety and nutritional values. Herbs have been used in foods since ancient times, not only as folk medicine, but also as flavoring agents and food preservatives (Deepa *et al.,* 2013). Therefore, the present study investigates the effect of plant extracts on food spoilage microbes.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

Dacryodes edulis was 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 for 130 hours. Thereafter the leaves were cut into small sizes up to 1cm long and further dried up. Fully dried leaves were grinded into powder using kitchen grinder.

Plant Extraction

The distilled water, hot water and methanol extraction procedure was carried out according to the modified method of Qasem and Abu-Irmaileh (1985).

Distilled water extraction

20 g of powdered sample was soaked in 100 ml of distilled water within 1 liter conical flask. After that they were kept on a mechanical shaker for 24 hours and filtered through cotton cloth. The supernatant was centrifuged at 10,000 rpm for 5 minutes in order to separate the extra debris from the solution, which was served as a stock solution (5%) for aqueous extract. From that solution various concentrations of extract were prepared by the way of dilution.

Hot water extraction

20 g of powdered sample was soaked in 100 ml of distilled water within 1liter conical flask, thereafter the mixture was boiled and kept on a mechanical shaker for 24 hours and filtered through cotton cloth. The supernatant was centrifuged at 10,000 rpm for 5 minutes for separating the extra debris from the solution, which was served as a stock solution (100%) for aqueous extract. From that solution various concentrations of extract were prepared by the way of dilution.

Methanol extraction

For the methanol extraction, 10 g of the powdered sample was soaked in 100 ml of methanol for 72 hours in 500 ml conical flask and mouth stiff up by using aluminum foil. After 3 days the methanol solution was filtered by using muslin cloth within another conical flask. The supernatant was centrifuged at 10,000 rpm for 5 minutes for separating the debris from the solution. Afterwards the solution was poured into various petri dishes and left to get dried. After they were properly dried, they were scraped from the petri dishes into conical flasks and then 100 ml of distilled water was added. This was then used as stock for the preparation of the various concentrations of solution by way of water dilution.

Phytochemical Screening of the Plant Extract

Phytochemical screening for alkaloids, phenols, tannins, flavonoids, saponins, terpenoids, quinones and glycosides will be carried out according to the methods of Sofowora (2008)

Test Organisms

Bacterial and Fungal isolates were obtained from spoilt beef and chicken using standard microbiological techniques as described by (Cheesbrough, 2010) with modifications. The bacteria were grown at 37^{o}C on nutrient agar. After 24 hours, sub culture was done using Nutrient agar and Mac Conkey agar. The fungus was grown at 28±2 °C on potato dextrose agar (PDA). Spores of the fungus were collected from cultures on agar plates after 7days. Cultural and morphological identification as well as biochemical characterization of the isolates using standard protocols including Catalase test, Oxidase test, Coagulase test, Citrate test (Simmons' citrate Agar), Methyl Red/ Voges-Proskeur (MR/VP) test, Urease test, Indole test, Motility test, and Sugar fermentation test (Using Triple Sugar Iron Agar) were carried out. Pure cultures of the isolates were maintained in appropriate media for future use.

Screening for Antifungal Activities

Agar well diffusion method was used to screen the antifungal activities of different solvent extracts as displayed by (Daoud *et al*., 2015). 500 mls of Muller Hinton agar was prepared and poured into various sterile Petri dishes, the plates were then left to cool, there after they were dried using the oven. After drying the plate, a loopful of the inoculum was inoculated in the already prepared agar using the spread plate method. Thereafter, wells were made using a sterile cork borer (6 mm in diameter) into agar plates containing inoculums. Then, plant extract based on their concentration (100%, 75%) was decanted into the bored wells aseptically. Then, the plates were incubated at 37°C for 24 hours. Antimicrobial activity was detected.

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Statistical Analysis

The data obtained were analyzed by factorial Analysis of Variance (ANOVA) to determine significant ($P \leq 0.05$) effects. The significant differences between means were determined using Duncan's Multiple Range Test (DMRT). The results of the study were presented as Mean ± standard error of the trials in Tables 2 and 3.

RESULTS AND DISCUSSION

Several researchers have reported that plants contain bioactive substances (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. The methanol extract and distilled water extract has more phytochemicals than the hot water extract (Table1)

The methanol extract and distilled water extracts were more effective against all the microorganisms compared to the distilled water extract. The highest zone of inhibition of 31.50 ± 2.50 mm in distilled water extract was observed against *Trycophyton* sp. at 100 % while *Salmonella enterica* showed the highest zone of inhibition of 17.50±2.50 in 100% distilled water extract in the case of antibacterial activities of the plant extract. All zones of inhibitions for all organisms were significantly different at different concentrations (Tables 2 and 3).

Food safety is a major concern for both consumers and food manufacturers alike. Despite the high degree of awareness of food preservation methods, the occurrence of disease outbreaks caused by foodborne pathogens and spoilage microorganisms in foods is still increasing (Meng and Doyle 1998). Foods need to be safe and fresh with prolonged shelf-life. In this study, the screening of the water and methanol extracts indicated the presence of glycosides, phenols, alkaloids, terpenoids, flavonoids and saponins. Several researchers investigated the efficiency of plant extracts and their effective compounds as antimicrobial agents to control growth of food borne and spoilage bacteria. Some researchers have suggested that antimicrobial components of the plant extracts (terpenoid, alkaloid and phenolic compounds) interact with enzymes and proteins of the microbial cell membrane causing its disruption to disperse a flux of protons towards cell exterior which induces cell death or may inhibit enzymes necessary for amino acids biosynthesis (Gill *et al.,* 2006).

The extracts were effective against all the microorganisms studied. The extract showed antibacterial effects against *Proteus vulgaris, Salmonella enterica* and *Citrobacter freundii.* Among the bacteria species, *Salmonella enterica* was more susceptible to *Dacryodes edulis* than other species like *Proteus vulgaris and Citrobacter feundii.* The plant extract had more of antifungal effect than antibacterial effect. These results are in accordance with that of Mahfuzul *et al.* (2007) and Pandey *et al.* (2011). 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.

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).

CONCLUSIONS

This study was aimed to examine the antimicrobial effect of extracts from *Dacryodes edulis.* Ten grams of *Dacryodes edulis* was extracted in 100 ml of distilled water, hot water and methanol. 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. The phytochemical screening was carried out to check for the bioactive compounds present in the plant extract. The isolation of the organisms from the spoilt beef and chicken was done using standard method. Thereafter, the organisms were sub-cultured in other to obtain a pure culture. The identified organisms are *Salmonella enterica, Proteus vulgaris, Citrobacter freundii, Trycophyton*

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Table 1. Phytochemical screening of *Dacryodes edulis*

Phytochemicals	Distilled Water Extract	Hot Water Extract	Methanol Extract
Alkaloids	-	+	+
Phenol	+	+	+
Glycoside	+	+	+
Flavonoid	-	-	+
Terpenoid	+	-	-
Saponin	+	+	+
Quinon	+	+	+
Antraquinon	+	-	+
Steroid	+	-	-
Phylobatanin	-	-	-

+ indicates the presence - indicates the absence

Table 2. Antifungal effects of *Dacryodes edulis* crude extracts against *Trycophyton* sp and *Cladosporium* sp

Extract	Concentration	*Trycophyton sp*	*Cladosporium sp*
Distilled water	100%	31.50±2.50	23.50±3.50
	75%	27.50±2.50	20.00±5.00
Hot water	100%	10.00±0.00	18.50±6.50
	75%	10.00±0.00	14.00±4.00
Methanol	100%	17.50±2.50	11.00±1.00
	75%	14.50±2.50	10.50±0.50
		-	

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Table 3: Antibacterial effect of *Dacryodes edulis* crude extracts against *Proteus vulgaris, Citrobacter freundii* and *Salmonella enterica*

Extract	Concentration	*Proteus vulgaris*	*Citrobacter*	*Salmonella enterica*
Distilled	100%	16.50±1.50	12.50±2.50	17.50±2.50
	75%	14.00±0.00	12.50±2.50	13.50±0.50
Hot water	100%	10.50±1.50	-	11.50±1.50
	75%	9.50±0.50	-	10.00±0.00
Methanol	100%	12.50±2.50	15.00±3.00	12.00±0.00
	75%	11.00±1.50	12.50±2.50	10.00±0.00
		-		

and Cladosporium. These organisms were then subjected to antimicrobial activity of the methanol and aqueous extract using the agar well diffusion method. The results revealed that the distilled water extract and methanol extract of the leaves of *Dacryodes edulis* showed a distinct degree of activities against bacterial and fungal spoilage organisms. The inhibition of microbial isolate by the extracts suggests that the extract of *Dacryodes edulis* have potential antibacterial and antifungal effects which can be adequately explored further in food preservative preparation.

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