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Physicochemical analysis and antimicrobial activity of modified and unmodified forms of extracts of *Dialium guineense* (Velvet tamarind) seed

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Abstract: This study deals with the antimicrobial activities of the n-hexane and ethanol extracts of *Dialium gineense* (black velvet tamarind) seed, its methyl and ethyl esters, against seven isolates: three gram-positive, three gram-negative and a fungus. The gram-positive isolates were *Staphylococcus aureus, Bacillus cerus CF7* and *Bacillus thuringensis* strain EB-151while the gram-negative isolates were *Pseudomonas aeruginosa* strain PGI, *Pseudomonas aeruginosa* strain 33k55 and *Escherichia coli*. The fungus was *Candida albican*. The result revealed that the n-hexane extract (i.e. oil), ethanol extract, the methyl and ethyl esters of the velvet tamarind seed do not possess antimicrobial activities on the tested organisms. The physicochemical properties of the n-hexane extract (the velvet tamarind seed oil) were determined and it was found that the oil will be good for culinary purposes (due to its acid value), in making shampoos and leather shaving creams (due to its saponification value).

Keywords: *Antibacterial; Antifungal; Esterification; Extraction; Modification*

1. Introduction

All through history, irrespective of culture and social strata, plants have been a dependable source of medicine, the manner of usage ranging from boiling the plant part, soaking in appropriate solvent, grinding into powder, as additives to meal and bath water etc. to the extraction and isolation of the pharmacological active compounds in various plant parts (Newman and Craig, 2007; Bouyanzer *et al.*, 2017; Taibi *et al.*, 2023; Haddou *et al.*, 2023). It is important to note that there are a lot of plant-originated drugs in clinical medicine and those products have in turn become one of the most important resources for developing new lead compounds and molecular scaffolds (Hawas *et al.*, 2012). According to a research conducted by the World Health Organisation (WHO), about 80% of the world population depends on medicine from natural source (WHO, 2002). Studies also showed that about 121 drugs prescribed in the USA today are of natural source and about 90 of these are directly or indirectly from plant sources, and over 47% of the anticancer drugs in the market are derived from natural products or are natural product mimics (Hawas *et al.*, 2012). *Velvet tamarind* is one of such medicinal plants that have been utilized over time, especially in traditional medicine. Various parts of this plant, ranging from the root, bark, stem, leaves, pulp and seeds have been used in traditional medicine (Abiodun *et al.*, 2012; Mahraz *et al.*, 2024). It is found virtually in all tropical climate regions, from India through Africa, to the Caribbean and South America and up to South Florida, and its use are as varied as the culture that use it. In fact, it is hard to ascertain which use is more important: as food and beverage or as folklore medicine. But in Africa sub region, including Nigeria, it is widely used as both food and medicine (Mohamed *et al.*, 2015). *Dialium*

guineense is popularly called "Awin" by the Yoruba people, "Icheku" by the Igbos and "Tsamyar Kurm" by the Hausa speaking people of Nigeria (Ogu *et al.*, 2013). The tree grows to about 30 m high with low branching, seldomly straight, with a compartment of densely leafy crown with hairy leaves and white flower that produces the black velvet fruits. The fruits are almost circular and flattened, enclosing dry, light brownish sweet acidic edible pulp (Ogu *et al.*, 2013). Every fruit contains at most two seeds which are flat, circular, dark-brown in colour, with a width of about 3 mm thick. The seeds are shinny and coated with a thin layer of starchy material (Arogba *et al.*, 2006). They are hard to crush. Velvet tamarind seeds are underutilized as an abundant and cheaply available by-product of the tamarind pulp industry. Although there are many uses of this seed, very few have been utilized (Abiodun *et al.*, 2012). Among 109 new drugs approved in the period of 1981-2006, 69% are obtained from natural products (Ogu *et al*, 2013). Among the known plant species on earth; estimated at 250,000 – 500,000 plant species, only a small fraction has been experimentally scrutinized for the presence of antibacterial substances (Nair and Chanda, 2007). Although synthetic chemistry has provided new bioactive compounds/substances and the use of combinatorial techniques have increased considerably the number of compounds available for test, still there are high number of natural products and their derivatives among the best-selling drugs and yet, there are many natural products relatively untapped (Olajubu *et al.*, 2012). It is therefore the aim of this study to determine the antimicrobial activity of the n-hexane and ethanol extracts of *Dialium guineense* seeds, the methyl and ethyl esters of its oil on seven selected organisms. The physico-chemical properties of the seed oil will also be determined to assess other industrial relevance of the seed oil.

2. Methodology

2.1 Sourcing and preparation of samples

The fruits of black velvet tamarind used for this research were gotten from the Mile 1 fruit market in Port Harcourt, Rivers State. The shell of the fruit was manually removed and the seeds washed in clean tap water to remove the pulp and air dried. The seeds were further dried in the oven at 60^0C, then crushed to fine powder using grinding machine.

Fig. 1: *Dalium guineense* (Black velvet tamarind) fruits and seeds

2.2 Experiments

2.2.1 Determination of moisture content

The ground *Dialium guineense* seeds (2.0 g) was weighed and dried in the oven at 60^0C for 30 minutes and then re-weighed at regular intervals until a constant weight was obtained. The moisture content was calculated as given below:

$$\%\ Moisture\ content = \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 10$$

2.2.2 Extraction of velvet tamarind seed oil

The fine powdered *Dialium guineense* seeds (140.2 g) was packed into a thimble and placed in a Soxhlet extractor. The solvent (n-hexane) contained in a 500 ml heating flask was heated at reflux for 8 hours until all the oil was extracted from the powder. When the extraction was done, the mixture of n-hexane and the oil was filtered to remove any solid particle from the solution. Finally, the solvent was removed to afford the oil and its weight recorded. The residue obtained after the Soxhlet extraction (i.e. ground velvet tamarind seed deplete of the oil) was air-dried to remove traces of hexane and stored for further usage.

2.2.3 Determination of percentage oil yield

The weight of the oil as obtained in the "extraction of velvet tamarind seed oil" above was the actual yield. The percentage yield was obtained by dividing the weight of oil in grams by weight of dried seed multiplied by 100 as given the equation:

$$\%\, Weight\ of\ oil = \frac{weight\ of\ oil\ \times 100}{weight\ of\ sample}$$

2.2.4 Determination of saponification value

The extracted oil (0.5 g) was weighed into a flask and 12.5 ml of 0.5 N ethanolic potassium hydroxide solution was introduced into the oil. The mixture was heated at reflux in a water bath and with stirring for 30 minutes. 0.5 ml of phenolphthalein indicator was then added and the hot solution was allowed to cool and later titrated against 0.5 N hydrochloric acid. The volume of the acid used was recorded. A blank titration was carried out using the same procedure and saponification value calculated as:

$$Saponification\ value = \frac{56.1(V1-V2)N}{Wt}$$

where N = Normality of Hydrochloric acid; V1 = Volume of HCl used in the test; V2 = Volume of HCl used in the blank; Wt = Weight of the oil used.

2.2.5 Determination of peroxide value

The oil (0.5 g) was measured into a flask and dissolved in 15 ml acetic acid/chloroform (3:2) solution and then heated in a water bath for 30 seconds with continuous swirling. 0.5 ml saturated potassium iodide solution was added followed by 15 ml distilled water. The resultant mixture was then titrated with 0.01 N $Na_2S_2O_3$ solution with constant and vigorous swirling until the yellow colour disappears. 0.05 ml of 1% starch solution was then added which turned the mixture blue, the titration continued until the blue colour disappeared at end point and the peroxide value calculated as shown below.

$$Peroxide\ value(meq/1000g) = \frac{Titre\ (ml)\times N\times 1000}{Weight\ of\ oil}$$

where N = Normality of $Na_2S_2O_3$.

2.2.6 Determination of acid value

The oil sample (0.5 g) was weighed into a 250 ml flask containing a mixture of 12.5 ml ether and ethanol in the ratio 1:1 and 0.5 ml phenolphthalein indicator. This was then titrated with 0.5 N NaOH

solution, until a pink colour was observed at the end point. A blank titration was conducted following the same procedure and the acid value calculated as:

$$Acid\ value = \frac{Titre(ml) \times 56.1 \times N}{Weight\ of\ oil}$$

where N = Normality of the potassium hydroxide or sodium hydroxide.

2.2.7 Determination of iodine value

Velvet tamarind seed oil (0.2 g) was weighed into a flask and Wiji's solution (i.e. iodine in glacial acetic acid) was then added. A blank was also prepared. Both mixtures were then kept in a dark place for 30 minutes at ambient temperature. Thereafter, 15 ml of a solution of potassium iodide was added, followed by 100 ml of distilled water. The mixture containing the oil was then titrated against 0.10 M $Na_2S_2O_3$ solution with constant swirling using 2 ml of 1% starch solution as the indicator until the blue colour disappears indicating the end point. A blank titration was conducted following the same procedure and the iodine value calculated as:

$$Iodine\ value = \frac{12.692\ (Tb - Ts) \times N}{Wt}$$

where N = Normality of $Na_2S_2O_3$, Tb = titre value of the blank and Ts = titre value of the sample.

2.3 Preparation of methyl ester

KOH pellet (0.4 g) was weighed into a conical flask and sealed using a foil. 20 ml of anhydrous CH_3OH was added to the KOH in the flask and stirred using a magnetic stir bar until it dissolved. 10 ml of *Dialium guineensis* seed oil was measured into another flask; another stir bar was introduced and then heated to 55^0C. The mixture in the first flask (methoxide) was then poured into the flask containing the heated oil. The resulting mixture was refluxed for 1 hour while being stirred at 50^0C. Thereafter, the mixture was transferred into a separatory funnel and allowed to stand till the next day. It was then washed with distilled water (10 ml × 3). CH_2Cl_2 (10 ml) was added to the mixture and filtered through a short column of anhydrous Na_2SO_4 and the dichloromethane allowed to evaporate to recover the fatty acid methyl esters (FAMEs). The resultant mixture was allowed to stand, thus separating into two layers, the lower layer was drained off into a 10 ml beaker, dried with anhydrous Na_2SO_4 and left open for the chloroform to evaporate. The final product is weighed, stored in a sample bottle and labelled as methyl ester.

2.4 Preparation of ethyl ester

KOH pellet (0.4 g) was weighed into a conical flask and sealed using a foil. 20 ml anhydrous ethanol was added to the KOH pellets, stirred using a magnetic stir bar. The same procedure used in the preparation of methyl ester above was then followed. The product got is weighed, stored in a sample bottle and labelled ethyl ester.

2.5 Determination of antimicrobial activity

The determination of antimicrobial activity was carried out at the microbiology laboratory of the University of Port Harcourt, Rivers State. It was carried out on eight samples: the methyl ester, ethyl ester, velvet tamarind seed oil, ethanol extracts, ethanol, ampicillin and griseofulvin (ampicillin, griseofulvin and ethanol were used as standards) using the well agar method. The already characterized

bacteria; *Pseudomonas aeruginosa* strain PG, *Pseudomonas aeruginosa* strain 33K55, *Escherichia coli* (all gram negative), *Bacillus thuringensis* strain EB-151, *Bacillus cereus* strain CF7, *Staphylococcus aureus* (gram positive), and *Candida albican* (a fungus) were resuscitated in freshly prepared nutrient agar plate from which a McFarland standard was prepared. The sample concentration (200 – 250 mg/ml) using 30% DMSO were prepared as described by Akujobi *et al.* (2004). 0.10 ml of each bacterium from the standard inoculum of each specie bacteria were dispensed into sterile petri-dishes followed by 200 ml molten Miller Hinton agar (at 40 – 45^{0}C) and gentle swirling for a uniform spreading of the bacterial colonies. The content of the plates was allowed to cool and solidify. This was followed by five holes being bored in each plate. The right concentrations of each sample were dispensed into well-labelled wells and incubated at 35^{0}C for 18 – 24 hours. The zones of inhibition were measured in ml and compared with those of the standards.

3. Results and Discussion

3.1 Physicochemical properties

The yields and physicochemical properties of Dialium guineense (velvet tamarind) seed oil and its methyl and ethyl esters were as summarized in Table 1. *Dalium guineense* seed oil is amber-green coloured oil with a very pleasant smell and viscous texture. The viscosity of a substance is been known to depend on the substances' inner molecular structure. The viscosity of this oil makes it a good lubricant: the greater the fluids viscosity, the greater the load it can carry. The emollient property of this oil also makes it a good natural moisturizer for the skin. The oil yield of a seed is the quantity of oil in the seed of that plant. The oil yield of velvet tamarind seed is 4.35%, lower than most seeds: palm kernel (45.61%), breadfruit (9.71%), groundnut oil (35.75%), African bean (12.23%). This is low to be considered an oil seed for commercial purposes but could be considered for pharmaceutical and other industrial purposes (Gupta *et al*., 2014). The acid value of velvet tamarind seed oil is 1.38 mg KOH/g. This is low compared to those of melon seed oil (3.5 mg KOH/g), almond oil (7.6 mg KOH/g), and fluted pumpkin oil (3.5 mg KOH/g). Since the acid value is a representation of the amount of free fatty acid in an oil as a result of enzymatic activity and an indication of oil spoilage, *Dalium guineense* seed oil can withstand spoilage; and since the pulp and the seed are edible, it can be used for culinary purposes. The maximum acceptable level of acid value for edible oil is 4.0 mg KOH/g (CODEX Alimentarius Commission, 1982), for edible oils. Acid values can be used to check the level of deterioration of the oil. Saponification value is inversely proportional to the average molecular weight of the fatty acids presents in the oil. Consequently, oils with high saponification value contain higher amount of lower molecular weight fatty acids. The saponification value of *Dialium guineensis* seed oil is 328.185 mg KOH/g higher than those of palm kernel oil (214.71 mg KOH/g), breadfruit (221.59 mg KOH/g), groundnut oil (198.63 mg KOH/g) and coconut oil (191.1 mg KOH/g) (Oluma *et al*., 2008). Oils with saponification value of 200 mg KOH/g and above have been reported to possess low molecular weight (Abayeh *et al*., 1998); this suggests that tamarind seed oil contains predominantly fatty acids that have low molecular weight. Hence, could be applied in soap making, production of shampoos and leather shaving creams. The iodine value of *Dialium guineensis* seed oil is 13.96 mgI_2/100g. This is higher than those of palm kernel oil (3.927 mgI_2/100g) but below those reported for bread fruit oil, groundnut oil, pumpkin seed oil, African bean oil, and melon seed oil which are 64.97 mgI_2/100g, 77.11 mgI_2/100g, 84.1 mgI_2/100g, 86.31 mgI_2/100g and 126.90 mgI_2/100g respectively. Iodine value is a measurement of the average degree of unsaturation in oil. The iodine value of *Dialium guineensis* seed oil; 13.96 mgI_2/100g is between those of those of coconut oil (10 mgI_2/100g) and palm kernel oil (37 mgI_2/100g) and both oils contain saturated fatty acids predominantly.

Table 1: The physico-chemical properties of *Dialium guineense* (velvet tamarind) seed oil and its methyl and ethyl esters

S/N	Parameters	Values
1.	Colour	Amber-green
2.	State at room temperature	Viscous liquid
3.	Oil yield (%)	4.35
4.	Moisture content (%)	1.00
5.	Smell	Very pleasant
6.	Acid value (mgKOH/g)	1.38
7.	Saponification value (mgKOH/g)	328.18
8.	Iodine value (mgI_2/100g)	13.96
9.	Peroxide value (mg Equiv.O_2/kg)	4.00
10.	Ethyl ester yield (%)	12.00
11.	Methyl ester yield (%)	22.00

Consequently, the iodine value of *Dialium guineensis* seed oil suggests that it contains more of saturated fatty acids. The low iodine value of this oil also makes it less prone to oxidation and polymerization, especially when heated thus making it very safe for cooking and can likely find its application in the industries for the production of vegetable-based ice cream except that it would not be commercially viable considering the oil yield of the seed. Furthermore, the iodine value indicates that this oil is non-drying oil as good drying oil oils have been reported to have iodine value above 100 mgI_2/100g (Eromosele *et al*., 1993). Peroxide value is an indicator of an oil's oxidative stability.

The low peroxide value of *Dialium guineensis* seed oil (4.0 mgEquiv.O_2/Kg) suggests that the oil is resistant to lipolytic hydrolysis and oxidative deterioration. Giving the "Codex Standards for Fats and Oils from Vegetable Sources", the maximum peroxide value for an oil suitable for human consumption is 10 milliequivalents of active oxygen/Kg oil. Therefore, the peroxide value of velvet tamarind seed oil is satisfactory and this means that the oil can be stored for a longer time without deterioration. Furthermore, the low peroxide value of this oil is expected since its iodine value is quite low (13.96 mgI_2/100g) indicating lower proportion of unsaturated fatty acids. Oxidation of an oil is influenced by the fatty acid composition of the oils: oils with more unsaturated fatty acids are oxidized more readily than those with less unsaturated fatty acids. In a study carried by Moronkola *et al* (2017) on chemical compositions of *Dialium guineense* Willd. leaf, stem-bark and fruit Essential Oils, the volatile essential oils were obtained by hydro-distillation, using an all-glass apparatus adapted to British Pharmacopeia specifications and gave good yields of 0.06 to 0.10%, which was studied using GC and GC-MS. The results of their study revealed that twenty out of twenty-five compounds, representing 92.91% of the leaf oil were characterized, while eighteen and thirteen out of twenty-one and eighteen compounds were identified in stem-bark and fruit oils, representing 85.65% and 81.44% of each respectively. Most abundant compounds were cis-3-hexenyl butanoate in leaf, and trans-δ-9-octadecenoic acid in both stem-bark, and fruit essential oils. Dominant classes of compounds (%) in leaf oil were esters (56.86), terpenoids (18.27) and acids (11.20); stem bark had mostly acids (57.41), esters (19.82) and terpenoids (3.05); most abundant in fruit oil were acids (65.79), hydrocarbons (8.56) and esters (3.42). According to Moronkola *et al* (2017), acids and esters dominated the 3 essential oils of *D. guineense*, with appreciable amounts of terpenoids and hydrocarbons in the three oils.

3.2 Esters yield

The methyl and ethyl esters yield of the transesterification of the velvet tamarind seed oil using base (KOH) catalysis at 55°C to 60°C was 22% and 12% respectively. This is higher than those of coconut

oil (10.4% ethyl ester) at same conditions (Ahmed *et al*., 2008) but very low when compared to those of palm oil (89% - 98% at temperature of 40ºC to 60ºC) the highest yield being at 60ºC for methyl ester (Onuekwusi *et al*., 2014). This suggests that *Dialium guineensis* seed oil may not be applied in the production of biodiesel considering its low oil and ester yield.

3.3 Antimicrobial activities

Plant oils have been known to owe their antimicrobial property not only to the presence of some fatty acids, but also to the presence of phytochemicals like the phenolic compounds and aromatic compounds likes terpenes and terpenoids (Sodha *et al*., 2015; Joseph *et al*., 2012). The results of the antimicrobial activity of *Dialium guineensis* seeds' oil and its methyl and ethyl esters, showed that there were no zones of inhibition on the selected isolates (figures 2 to 6).

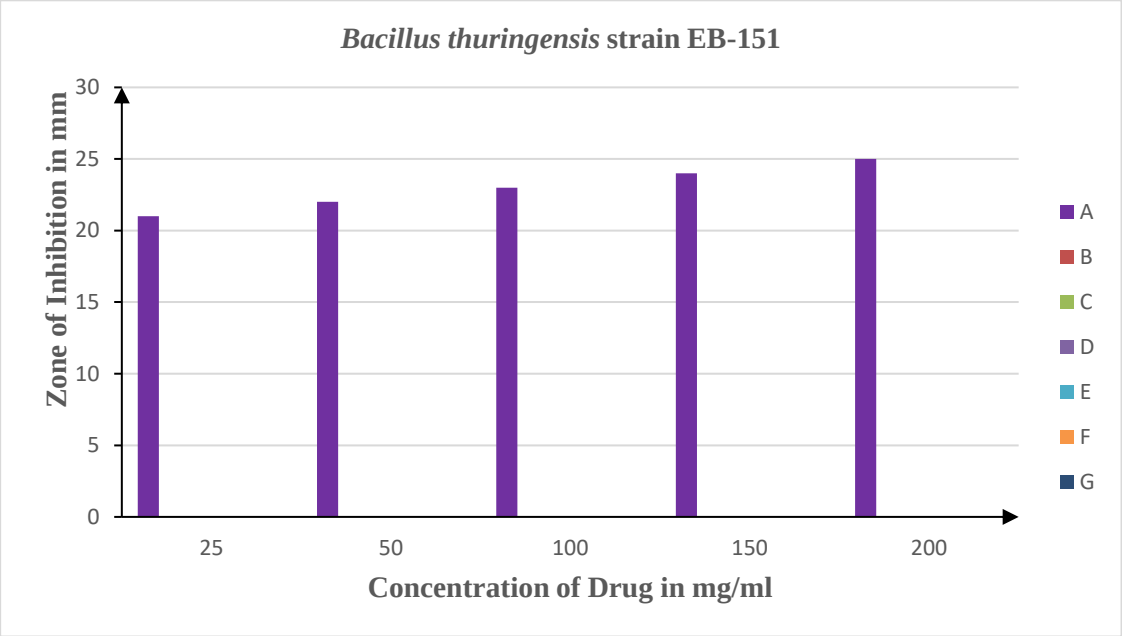

Fig. 2: Activity of drugs on *Bacillus thuringensis* strain EB-151

Note: A= Ampicillin for figures 1, 2, 3, 4 and is Griseofulvin for figure 5; B= Tamarind seed oil, C= Ethyl ester, D= Methyl ester, E= Ethanol extract of the defatted seed powder, F= Ethanol extract of the fresh seed powder, G= Ethanol solvent

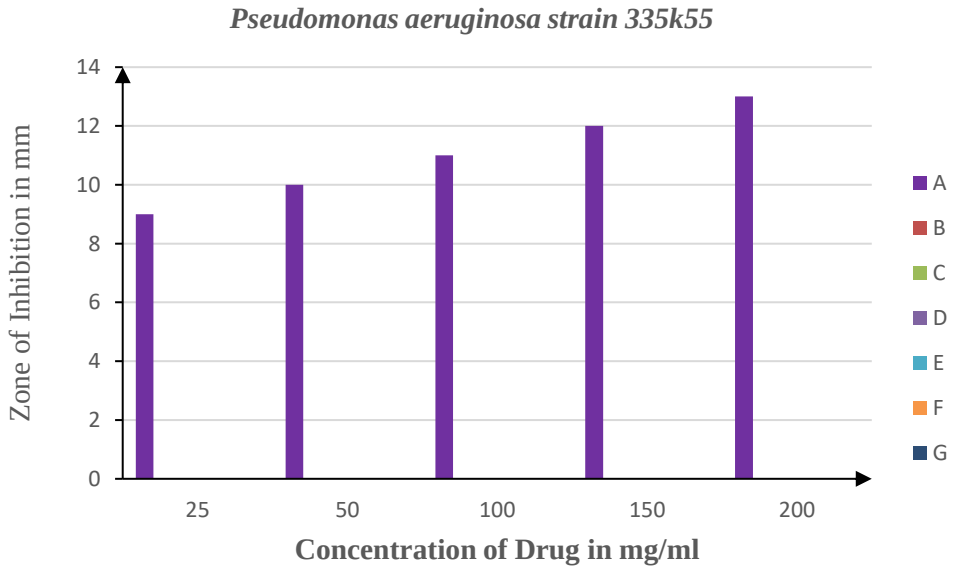

Fig. 3: Activity of drugs on *Pseudomonas aeruginosa strain 335k55*

It could be that the extracts are active on other isolates not tested in this study or that the extracts from other solvents not used in this study may be active on the tested isolates. Whatever is the case, the results of this study revealed that the n-hexane and ethanol extracts of velvet tamarind seed are not active on the isolates listed above. This may act as a guide to future researches on the choice of isolates and solvents in the search for bioactive substances from Dialium guineensis (velvet tamarind) seeds. The richness of natural plants in bioactive molecules as well as minerals as selenium favors their wide applications in several medical uses as antioxidant, anti-inflammatory, antibacterial, antifungal, and anticancer (Elbouzidi *et al*., 2024; Ogbuewu *et al*., 2023; Diass *et al*., 2023; Elmsellem *et al*., 2019).

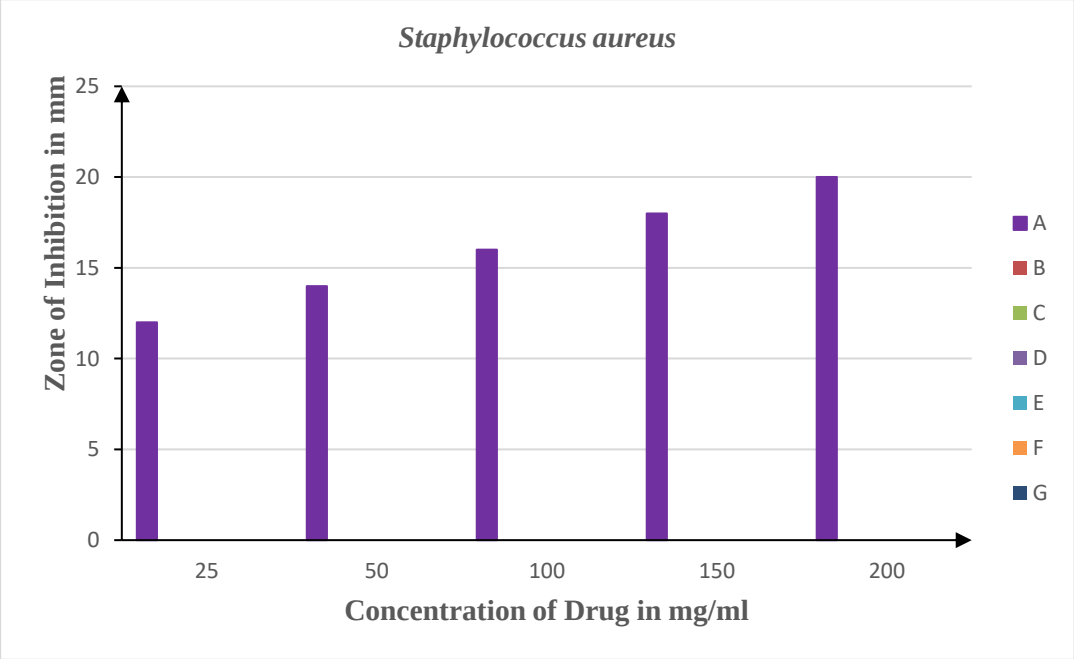

Fig. 4: Activity of drugs on *Staphylococcus aureus*

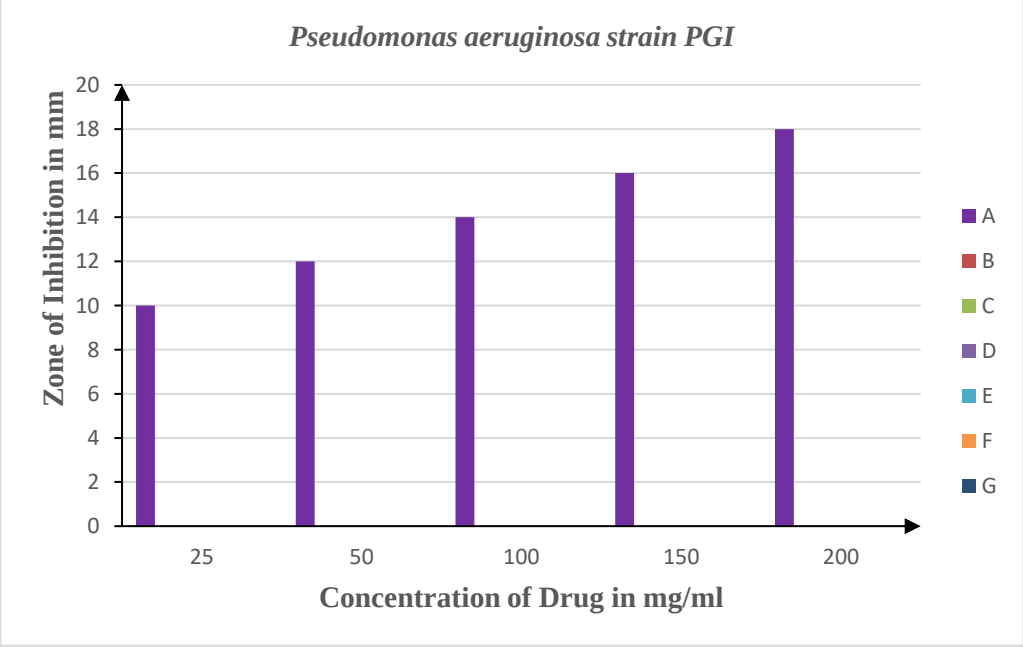

Fig. 5: Activity of drugs on *Pseudomonas aeruginosa strain PGI*

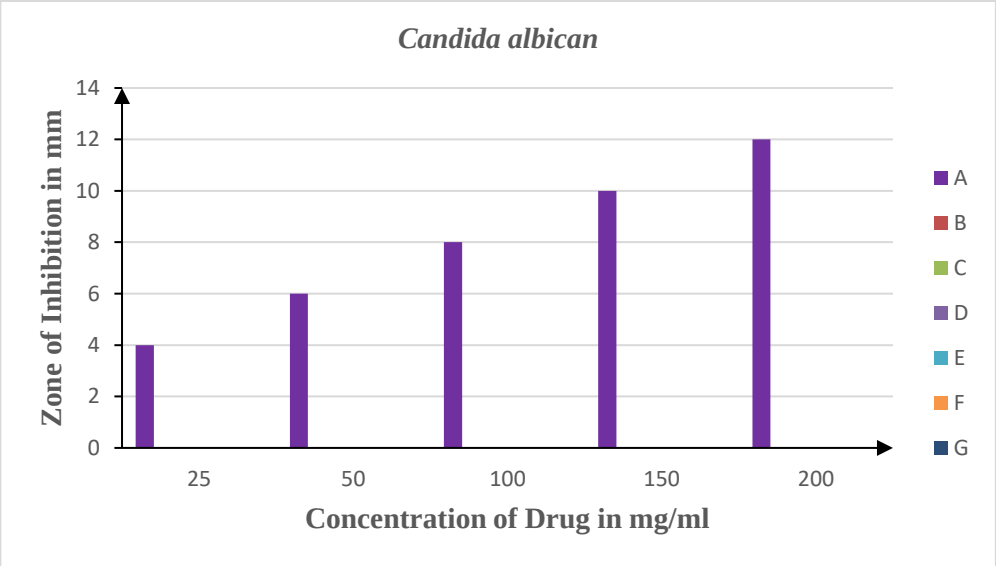

Fig. 6: Activity of drugs on *Candida albican*

Conclusion

The result of the analysis carried out on *Dialium guineensis* seed oil shows that it could find applications in culinary purposes, manufacturing of vegetable-based ice creams, manufacturing of soaps, shampoo, and lather shaving creams. However, the oil yield of *Dialium guineensis* seeds oil is quite low indicating that it is not economically viable as a source of vegetable oil. It is non-drying oil hence cannot be used to produce varnish nor paints. In addition, the oil's low iodine, saponification, and acid values indicate that the majority of its molecules are low molecular weight saturated fatty acids. Velvet tamarind seed oil would therefore be less susceptible to oxidation and degradation and could be kept in storage for an extended period of time without going bad. Furthermore, the antimicrobial activity carried out on the extracts (i.e. oil and ethanol extracts) and its methyl and ethyl esters against seven selected isolates: *Pseudomonas aeruginosa* strain PG, *Pseudomonas aeruginosa* strain 33K55, *Escherichia coli* (all gram negative), *Bacillus thuringensis* strain EB-151, *Bacillus cereus* strain CF7, *Staphylococcus aureus* (gram positive), and *Candida albican* (a fungus), using Amoxicillin, Griseofulvin and ethanol as standards, showed no zones of inhibition suggesting that they had no antimicrobial activity on the selected isolates.

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Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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