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Antimicrobial Screening and Physicochemical Properties of the Seed Oil of *Adenopusbreviflorus*

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ABSTRACT
Adenopusbreviflorusfruits has a long indigenous medicinal history values for curative and preventive measures against viral and bacterial diseases such as measles, chicken pox and enteritis. This study investigated the physicochemical properties and antimicrobial potential of A. breviflorusseed oil against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli with the aim to facilitate its utilization as essential oil. Extraction of oil was done with soxhlet apparatus using petroleum ether as solvent. Results revealed that the colour of seed oil was yellow with Iodine value of 112.8wijs. Refractive index was 1.482, while Acid and Peroxide value was 5.32 mgKOH/g and 7.29 29 MeqO_2/Kgrespectively. Antimicrobial screening using agar well diffusion method on the microorganisms showed no inhibition against the organisms except Pseudomonas aeruginosa with zone of inhibition of 11.17±2.38 mm. Therefore, the seed oil of A. breviflorus can be explore for domestic and industrial purposes but not in the treatment of infectious diseases caused by Staphylococcus aureus,Bacillus subtilis, Klebsiella pneumoniae and Escherichia coli.
Keywords: *Adenopusbreviflorus, Antimicrobial Screening, Physicochemical Properties, Seed oil*

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I. INTRODUCTION

The continuous need to discover new antimicrobial compounds with novel chemical structure and mechanisms of action for new and re-emerging infectious diseases has been emphasized by several authors. [7]reported that researchers are increasingly turning their attention to folk medicine in search of better drugs against microbial infections. Many infectious diseases have been known to be treated with herbal remedies and many natural products either as pure compounds or as standardized plant extracts provide opportunity for new drug because of their chemical constituents.Oil from parts of plants has been shown to exhibit antifungal activity against a wide range of pathogenic fungi and bacteria species [2, 3, 9, 10, 12]. Oil of plant origin has also been used for various cosmetic purposes particularly in the formulation of skin and body care products depending on their source and constituents[13]. Plant oils represent one of the important key materials that can be obtained from biomass cheaply and processed readily to supply the appropriate and important raw materials for the chemical and allied industries [9].

*Adenopusbreviflorus*Benth. (Common name – wild colocynth; Local name - tagiri)is a wild species belonging to the family Cucurbitaceae.Some species in the family possess seeds which are sources of oils that are traditionally used as food and are considered safe for human consumption. The fruit of*A. breviflorus*have been claimed in folk traditional literature to be valuable against a wide variety of diseases in man especially in infants and growing children[11]. Antimicrobial potentiality of different extract of *A.breviflorus*fruitshas been extensively studied[6, 15]and[14]reported that the fruit is widely used in folklore medicine in West Africa as herbal remedy for the treatment of measles, digestive disorders, and as wound antiseptics (e.g. umbilical incision wound) in man, while the livestock farmers use it for the treatment of Newcastle disease and coccidiosis in various animal species, especially poultry. However, despite the acclaimed and documented uses, there appears to be a paucity of information from literature on the antimicrobial effects ofthe seed oils. In this work, we report the physicochemical properties and antimicrobial activities of seed oil of *A. breviflorus* on some microorganisms that are human pathogens including food borne, intestinal, pathogens and dermatophytes.

II. MATERIALS AND METHODS

Collection of sample fruits

Mature fruits of *A. breviflorus*were collected from the Biological Garden of the Department of Science Laboratory Technology, Moshood Abiola Polytechnic Abeokuta. The fruits were cut open into two and the seeds extracted. The extracted seeds were sun-dried for 5-7 days and later in a hot-air oven at 105 °C for 1 hour.

The dried seeds were de-shelled, blended into flour which was put into a plastic container with cover and stored in a refrigerator for further use.

Extraction of oil

150 g of the seed flour was placed in the thimble of a Soxhlet apparatus and extracted with diethyl ether (Analytical grade). The oil extracted was separated from the solvent by evaporation and quantified gravimetrically.

Collection of Test Organisms

The test organisms that were used for this study were all clinical isolates obtained from Sacred Heart Hospital, Lantoro Abeokuta, Ogun state Nigeria. They are*Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis* and*Klebsiella pneumoniae.*

Antibacterial screening of oil

The antibacterial activity of the oil was determined by modified agar-well diffusion method described by [4]. Nutrient agar plates were seeded with 0.2 mL of 24 h broth cultures of each isolate. The plates were allowed to dry for 1 h. A sterile cork-borer (6 mm diameter) was used to cut wells in each of the plates; 1 ml of the oil was introduced in the well while Control experiments were also carried out where the holes were filled with 200 mg of Ciprofloxacin as positive control. Each extract was tested in duplicates. The agar plates were left on the bench for 1 hour for proper diffusion of the extracts and later incubated at 37^0C for 24 hours. Antimicrobial activity was determined by measuring the diameter of zone of inhibition (mm).

Determination of physicochemical properties of the oil

The physical state of the oil at room temperature were determined through visual inspection. The refractive index, free fatty acid, specific gravity, peroxide value, saponification value, unsaponification matter, acid value, iodine value and percentage oil yield were determined according to the methods of [1].

III. RESULTS

Antimicrobial effects of oil on test organisms

Table 1 showed the effect of *A. breviflorus*seed oil on the test organisms. It was observed that the oil exhibited no activity against *Staphylococcus aureus,Bacillus subtilis, Klebsiella pneumoniae* and *Escherichia coli.* However, the oil was active against *Pseudomonas aeruginosa* with 11.17±2.38mm as zone of inhibition. Ciprofloxacin (200mg) used as a positive control also had varying activities against the test organisms with a highest zone of inhibition of 8.50±2.17 mm against *Pseudomonas aeruginosa*, followed by7.83±2.85 mm against *Klebsiella pneumonia* while the least zone of inhibition of 3.50±1.08mm of the control was against *Bacillus subtilis.*

Table 1: Antimicrobial effects of oil on test organisms

Organism	Zone of inhibition (mm)	Control (mm)
Staphylococcus aureus	NI	5.83±1.64
Bacillus subtilis	NI	3.50±1.08
Pseudomonas aeruginosa	11.17±2.38	8.50±2.17
Klebsiella pneumonia	NI	7.83±2.85
Escherichia coli	NI	4.50±1.92

Key:NI- No InhibitionControl – Ciprofloxacin (200mg)

Physicochemical properties of the seed oil

The oil is yellow in colour and the percentage yield is 52.09. Refractive index is 1.482% while Specific gravity recorded 0.938%, Acid, Saponification, Iodine and Peroxide valuesrecorded 5.32mgKOH/g, 219.3mgKOH/g, 112.8wijs and 7.29MeqO_2/Kg respectively (Table 2).

Parameters	Value
Colour	Yellow
Refractive index (%)	1.482
Specific gravity (%)	0.938
Acid value (mgKOH/g)	5.32
Iodine value (wijs)	112.8
Peroxide value (MeqO_2/Kg)	7.29

Free fatty Acid (%)	2.62
Saponification value (mgKOH/g)	219.3
Unsaponifacation matter (%)	1.61
Oil yield (%)	52.09

Table 2: Physicochemical properties of the seed oil

IV. DISCUSSION

The antibacterial screening of the seed oil of *A. breviflorus* against the test isolates showed that the oil was active against only one of the test organisms (*Pseudomonas aeruginosa*).The result also revealed that the oil hadhigher antibacterial potential against the organism than Ciprofloxacin (200mg) used as a positive control. Therefore, the oil could be used as alternative natural antibiotics against *Pseudomonas aeruginosa*.

The physicochemical properties of the oil presented in Table 2 revealed that the colour of the oil is yellow with refractive index of 1.482. Refractive index is used to measure the change in unsaturation as the fat or oil is hydrogenated [5]. The value obtained was above the value (1.45) reported by [11] and also above the value approved by [8] for named vegetable oils. However, [2] reported the refractive index value of 1.48 in *Citrullus lanatus* (Watermelon) which also belongs to the family Cucurbitaceae. Acid value obtained from this study higher than the accepted permissible values (4.0mg) for edible oils by [8].

The iodine value which indicates the level of unsaturation in oils showed that *A. breviflorus* seed oil contain high amount of poly-unsaturated fatty acids. The higher the iodine index, the more unsaturated the oil. Oils are also characterized into non-drying oils (<100), semi-drying oils (100-130) and drying oils (>130) based on the iodine value. The peroxide value recordedwas within the codex (1999) approved limit (<15Meq/kg) which signifies that that the oils are of good quality; the lower the peroxide value, the better the quality of the oil. The saponification value of 219.3 mgKOH/g obtained in this study was higher than the value 165.50 mgKOH/g and 175.0 mgKOH/gearlier reported by [16]and [11]respectively.

V. CONCLUSION

The seed oil of *A. breviflorus* can be explore as natural antibiotics against diseases caused by *Pseudomonas aeruginosa* and also for domestic and industrial purposes but not in the treatment of infectious diseases caused by *Staphylococcus aureus,Bacillus subtilis, Klebsiella pneumoniae* and *Escherichia coli.*

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