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Research Article

Antimicrobial Activities of Essential Oils from Safou (*Dacryodes edulis*) Grown in Côte d'Ivoire

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Abstract

Research on natural substances from plants contributes to the valorization of under-exploited and unknown plants in Côte d'Ivoire. Thus, the objective of this study was to highlight the antimicrobial properties of essential oils of Safou (*Dacryodes edulis*). To do this, essential oils (EOs) of Safou seeds and barks were extracted. Antimicrobial activity was determined on *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia*, *Proteus mirabilis, Aspergillus fumigatus* and *Candida albicans*. The minimum inhibitory dilution (MID) of the essential oils for each microorganism was also determined with 1/10; 1/25; 1/50 and 1/100 dilutions. Essential oils (EOs) from seeds and barks of Safou showed strong antimicrobial activity on the tested microorganisms. The inhibition diameters ranged from 12.5 to 27 mm. However, no inhibitory activity was observed on *Pseudomonas aeruginosa*. The minimum inhibitory dilution (MID) of Safou seed and bark EOs showed inhibition diameters ranged from 9 to 19.5 mm. EOs were more active on bacteria than on yeasts and molds. Safou essential oils could be potentially used as natural antimicrobial agents against infectious diseases in humans and for food preservation.

Keywords: *Safou (Dacryodes edulis); Safou seed; Safou bark; essential oil; antimicrobial activity.*

Introduction

Essential oils, also called plant essences, are products obtained from a natural raw material of plant origin. According ISO 9235 (2013), it is a product obtained from a natural raw material of vegetable origin, either by steam by steam distillation, or by mechanical processes from the epicarp of citrus fruits (citrus fruits), or by dry distillation, after separation of the possible aqueous phase by physical processes. They are aromatic, light and volatile, generally liquid at room temperature and insoluble in water. They are characterized by their odor, specific to the plants or plant organs from which they come. Biologically active plant extracts, including essential oils, are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential disease control agents. The secondary metabolites performs defensive role in plant from their invaders (Yami and Shukla, 2016). Approximately 3000 essential oils are known, of which 300 are commercially important especially in pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries. Essential oils or some of their components are used in perfumes and make-up products, as food preservers and additives, and as natural remedies in curing various ailments (Murthy. *et al.*, 2016). Aromatherapy is now considered to be another alternative way in healing people and the therapeutic values of aromatic plants lie in their volatile constituents such as

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monoterpenoids, sesquiterpenoids and phenolic compounds that produce a definite physiological action on the human body.

Among these biologically active plants is Safou (*Dacryodes edulis* (G. Don) H. J. Lam, Burseraceae), native to the countries of Central Africa and the Gulf of Guinea (Cameroon, Congo, Democratic Republic Democratic Republic of Congo, Gabon, Equatorial Guinea and Nigeria) (Aubréville, 1962). Different parts of the plant are used in different parts of Africa in the treatment of various ailments. The decoction of the leaves is employed to relieve certain disorders of the digestive tract, toothache and earache. The leaves and stem-barks are used to cure dysentery and anaemia (Ayuk. *et al*., 1999). The resin from the bark heals scars and other skin diseases in Nigeria. The leaves are also reported to be employed in the remedy of skin problems such as ringworm, scabies and rashes (Ajibesin, 2011). Several works have shown that *Dacryodes edulis* was one of the essential oil-bearing plants, rich in lipids, vitamins and minerals belonging to the Burseraceae family that had a long history in popular medicine (N'Doye, 2001). Tabuna and Kayitavu (2009) assert that it is possible to extract from the pulp and seed of Safou, food and cosmetic oil. In Côte d'Ivoire, the first Safou (*D. edulis*) trees were introduced before 1960s but disappeared (Bourdeaut, 1971). However, few scientific works have been published either on their nutritional and therapeutic potential. This study investigated the antimicrobial potential of essential oils from Safou seeds and bark (*Dacryodes edulis*) cultivated in Côte d'Ivoire necessary for their wider use.

Materials and Methods

Sample Collection

Dacryodes edulis fruits and bark (Safou) were harvested in the south of Côte d'Ivoire (Azaguié-blida, Agboville) from August to October 2021. The botanical authentication of this plant was done by the herbarium of National Floristic Center of University FELIX HOUPHOUET BOIGNY (Abidjan, Côte d'Ivoire), where a voucher specimen was conserved with reference number UCJ018940. The fruits were transported in net bags to Biocatalysis and Bioprocessing laboratory of NANGUI ABROGOUA University.

Essential Oils Extraction

The fruits were de-fleshed to separate the seed from the pulp. Then the seeds and bark were cut separately into small pieces and were thoroughly washed with sterilized water. Essential oils (EOs) were extracted from seeds and barks by hydrodistillation at atmospheric pressure using a Clevenger type apparatus (Clevenger, 1928). The plant material was placed in a still containing water and then boiled on a hot plate for 5 hours. The isolated fractions of plant parts exhibited two distinct layers an upper oily layer and the lower aqueous layer. Both the layers were separated and the essential oils were stored in clean glass vials after removing water traces

Extraction Yield

Extraction yield was expressed as a percentage of the initial mass of the fruit subjected to extraction (Stanojević. *et al*., 2009). The extraction yield is calculated according to the following formula:

$$\text{Extraction yield} = (\text{mass of essential oils} / \text{mass of fruit powder}) \times 100$$

Microbial Strains

Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis and *Aspergillus fumigatus, Candida albicans* were collected from Pasteur Institute of Côte d'Ivoire and Laboratory of Biotechnology and Food Microbiology of NANGUI ABROGOUA University respectively.

Preparation of the Microbial Suspensions

The density of selected organisms was adjusted equal to that of the 0.5 McFarland standards (1.5×10^8 CFU/mL) by adding them to nutrient broth for bacteria and Sabouraud broth for molds. A 24 hold culture was used for the preparation of microbial suspension. McFarland standards were used as a reference to adjust the turbidity of microbial suspension so that the number of microorganisms would be within a given rang.

Preparation of the Dilution Range

Because of the non-miscibility of essential oils with water, the series of dilutions were made in tween 80 in order to obtain solutions diluted at 1/10, 1/25; 1/50; 1/100 (Valgas. *et al*., 2007).

Antimicrobial Susceptibility Test

The National Committee for Clinical Laboratory Standards was the reference in determining antimicrobial susceptibility through the Diffusion Method. Different essential oils of Safou were tested using standard Mueller Hinton II plates to detect the antimicrobial activity of these testing solutions (Kirby and Bauer, 1960). Mueller Hinton agar and Sabouraud chloramphenicol agar were used to inoculate bacteria, yeasts, and molds respectively. The different agars were inundated with the different microbial suspensions prepared. Once the agars were dry, 6 mm diameter blotting paper discs were placed on the surface of the agars. The disks received 10 µL of the different essential oils. The inoculated Mueller Hinton and Sabouraud agars were incubated at 37°C for 24 hours and five days, respectively. Antibacterial and antifungal activities were evaluated by measuring the diameter of the zone of inhibition induced by the extracts with a graduated ruler. The assessment of the efficacy of the extracts was done according to the criterion of (**Poncé. *et al.*, 2003)**. Thus, a substance is said to be ineffective if the diameter of inhibition is less than 8 mm, whereas it is said to be effective if the diameter is between 9 and 14 mm. It is considered very effective when the diameter is between 15 and 19 mm and extremely effective if the diameter is greater than 20 mm.

Determination of the Minimum Inhibitory Dilution (MID)

MID determination was performed with a series of dilutions of the different essential oils 1/10, 1/25; 1/50; 1/100. The plates with inoculated microorganisms were provided with 10 µL aliquots in 6 mm diameter blotting paper discs. The MID was obtained from the highest dilution for which no bacterial and fungal colonies were obtained (Valgas. *et al*., 2007).

Statistical Analysis

The one-way analysis of variances (ANOVA) was carried out with the XLSTAT software to compare the variables measured on the different essential oils. This software was used to calculate the means and standard deviations of the analysis parameters.

Results

Physical Properties of Essential Oils of Safou (*Dacryodes edulis*) Seeds and Barks

Hydrodistillation of the Safou seeds and barks essential oil gave the limpid oil with a specific odour. Safou seeds gave the highest yield of 0.9 % compared to that of the Safou bark was 0.5 % based on the fresh weight of the sample.

Antimicrobial Activities of Essential Oils of Safou (*Dacryodes edulis*) Seeds and Barks

The results of the antifungal and antibacterial tests of the different essential oils (EOs) are recorded in Table 1. Antimicrobial activity of the seeds and barks essential oils was observed on *E. coli*, *S. aureus* and *C. albicans*. Only essential oils from seeds had antimicrobial activity on P. mirabilis and *A. fumigatus*. *K. pneumoniae* was inhibited by essential oils from the bark. No inhibitory activity was observed on *P. aeruginosa*. The inhibition diameters ranged from 12.5± 2.1 to 27.0± 4.2 mm. The highest inhibition diameter was recorded with *S. aureus* for seeds EOs (27± 4.2 mm).

Table 1: Antimicrobial activities of essential oils from seeds and barks of Safou (*Dacryodes edulis*)

	Essential oil of Safou seeds	Essential oil of Safou barks
Microorganisms	**Diameter of zones of inhibition (mm)**	
Escherichia coli	(22.0± 5.6)[a]	(16.0± 5.6)[b]
Staphylococcus aureus	(27.0± 4.2)[a]	(14.0± 2.8)[b]
Klebsiella pneumoniae	-	13.0±4.2
Proteus mirabilis	17.0± 1.4	-
Pseudomonas aeruginosa	-	-
Aspergillus fumigatus	12.5± 2.1	-
Candida albicans	(13 ± 4.2)[a]	(13±4.2)[a]

In line, the averages affected by the same letter are not significantly different at the 5% threshold according to the Newmann-Keuls test

Minimum Inhibitory Dilution of Essential Oils of Safou (*Dacryodes edulis*) Seeds and Barks

Table 2 shows minimum inhibitory dilution (MID) of essential oils from Safou seeds and barks. The inhibition diameters ranged from 9.0± 1.4 to 19.5± 7.7 mm. *E. coli* was the most sensitive bacterium among the tested microorganisms, with a minimum inhibition diameter of 9± 1.4 mm obtained with the 1/100 dilution for EOs of Safou barks. *A. fumigatus* was the most resistant microorganism among the tested microorganisms with an inhibition diameter of 9.5 ± 2.1 mm obtained with the 1/10 dilution for EOs of Safou seeds.

Table 2: Minimum inhibitory dilution of essential oils from seeds and barks of Safou (*Dacryodes edulis*)

	Huile essentielle de la graine				**Huile essentielle de l'écorce**			
	Diamètre d'inhibition en mm							
	1/10	**1/25**	**1/50**	**1/100**	**1/10**	**1/25**	**1/50**	**1/100**
Ec	(13,5± 2,1)[a]	(10,5±0,7)[ab]	-	-	(19,0 ± 1,4)[c]	(18,0± 2,8)[d]	(17,0± 4,2)[d]	(9,0 ± 1,4)[b]
Sa	(19,5± 7,7)[a]	(12,0±2,8)[b]	-	-	(12,5±3,5)[c]	(18,5± 3,5)[a]	(11,0±1,4)[b]	-
Kp	-	-	-	-	(12,5 ± 2,1)[a]	(11,5± 4,9)[a]	(9,0± 1,4)[b]	-
Pm	(17,0± 5,6)[a]	(9,0 ± 1,4)[b]	-	-	-	-	-	-
Af	9,5± 2,1	-	-	-	-	-	-	-
Ca	(13,0 ± 4,2)[a]	(9,5± 2,1)[b]	-	-	(18,0±2,8)[c]	(9,5± 2,1)[b]	(9,0± 1,4)[b]	-

In line, the averages affected by the same letter are not significantly different at the 5% threshold according to the Newmann-Keuls test. Ec : *Escherichia coli,* Sa : *Staphylococcus aureus,* Kp : *Klebsiella pneumoniae,* Pm : *Proteus mirabilis*, Af : *Aspergillus fumigatus,* Ca : *Candida albicans*

Discussion

The results of this study showed that yields varied depending on the plant organ. The yield of EO from seeds was higher than that of bark. This difference could be explained by the nature of the plant organ used and by the duration of extraction. Several authors such as (Quy. *et al.*, 2014); (Truong. *et al.*, 2019) showed that the efficiency of extraction was influenced by several parameters such as the nature of phytochemicals, the method used for extraction, temperature, extraction time, particle size as well as the solvent used.

This present study showed a strong antibacterial activity of Safou essential oils. The observed activity is probably due to the synergistic interaction between the compounds within the EO endowed with antibacterial activity just to cite hydrocarbon monoterpenes, oxygenated monoterpenes, (oxygenated) sesquiterpenes (Dorman and Deans, 2000). These compounds act by inducing membrane protein and lipid denaturation, inhibition of DNA replication and perturbation of membrane proton motive force, loss of energy substrate (glucose, ATP), leading directly to the lysis of bacteria (cytolysis) and therefore to its death (Riwom. *et al*., 2015). Another mechanism of action could be the inhibition of amylase and protease production which halts the toxin production by the bacteria, electron flow and result in coagulation of the bacterial cell content (Bakkali. *et al*., 2008; Nazzaro. *et al*., 2013). The results of this study are similar to those of Obame. *et al*., 2008; Ajebesin, 2011), (Riwom. *et al.,* 2015) and (Mordi. *et al*., 2019) who obtained antibacterial activities with the essential oils of Safou on the same bacteria tested in Nigeria and Gabon.

No inhibitory activity was observed with *Pseudomonas aeruginosa*. The resistance of this bacterium is due to the nature of its outer membrane that confers resistance to most biocidal agents. These results are similar to those of (Koudou. *et al*., 2008; Mordi. *et al*., 2019) who obtained no antibacterial activity on *P. aeruginosa* with essential oils of Safou in Gabon and Nigeria respectively.

Essential oils from seeds and barks have shown antifungal activity unlike other authors such as (Koudou. *et al.,* 2008) that is thought to be due to alterations in mold morphology resulting from disruption of cell division. However, in this study, the essential oils were more active on bacterial than fungal strains.

Conclusion

Essential oils from seeds and barks of Safou (*Dacryodes edulis*) showed a strong antimicrobial activity on microorganisms tested. All these results constitute a scientific justification for the use of essential oils of Safou (*Dacryodes edulis*) in traditional pharmacopoeia. These essential oils could potentially be used as natural antimicrobial agents against infectious diseases in humans and for the preservation of food products.

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