Biochemical properties of essential oil extracted from *Cyperus esculentus* L. (Cyperaceae) corm

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This study investigated the biochemical properties of the essential oil of the corm of *Cyperus esculentus*, as well as its antifungal activity on some dermatophytes and storage fungi. Also, the effect of the fungi on the biochemical properties of artificially-infected oil after 14 days of incubation was determined. The moisture content of the corm was 27.30 ± 0.41% and oil yield was 15.44 ± 0.38% g^{-1}. The essential oil was edible and non-rancid with free fatty acid value of 3.25 ± 0.27%; peroxide value of 5.26 ± 0.14 meq kg^{-1}, refractive index at 40°C of 1.47 ± 0.70, saponification value of 972.05 ± 26.81 mg KOH g^{-1}, and unsaponifiable matter 61.70 ± 1.32 g kg^{-1}. The fungi artificially inoculated on the oil changed its biochemical properties, turning the oil rancid. The oil showed significant antifungal activity, although below the 10 mm standard for a good inhibition except for *Trichophyton mentagrophytes* which was 11.29 ± 0.24 mm. The antifungal activity of the orthodox antibiotic, mycostatine, was significantly higher than that of the oil on the fungi tested.

Keywords: Biochemical property; Essential oil; *Cyperus esculentus* Corm; Antifungal activity

Cyperus esculentus L. (Tiger nuts) commonly called *Aya* in Hausa belongs to the family Cyperaceae. The plant is cultivated for its corm. *Cyperus esculentus* originates from the Mediterranean and western Asia. In Nigeria, it is cultivated in the northern part of the country (Dalziel, 1937). It adapts very well to a tropical environment. The fresh corm may be eaten raw if of high quality or it can be roasted in a pan with sand, in the same way the nuts of *Arachis hypogea* (groundnut) are roasted. From the roasted corm, a sweetmeat called *Dakuwa* in Hausa is commonly made. In Ghana, it is often cultivated on the coastal strip, and easily becomes naturalised. The corm is also common in the northern territories of Togo amongst some Togo tribes. There it is chiefly used uncooked and as a side-dish, or it may be used in the form of milky pap and taken as a food drink. In Sierra Leone, *C. esculeritus* corm is made into a type of chocolate drink. The corm can be used as a substitute for coffee, and for almonds in confectionary. *Cyperus esculentus* is known to have an aphrodisiac effect. It contains an edible oil similar in colour to olive oil (Burkhill, 1985).

To confirm the identity edibility of most oils and fats, it is normally considered sufficient to determine the iodine value, saponification value, unsaponifiable matter, free fatty acid (FFA) value, and peroxide value coupled with qualitative tests for appropriate adulterants (Hamilton and Rossell, 1986). The rancidity of the oil will also indicate the quality of the oil and affect its uses for soap, cream, and edibility. The FFA and peroxide value can be used to measure rancidity of the oil (Langanau, 1948; Kirk, 1991).

The aim of this study was to determine the biochemical properties of the essential oil extracted from *C. esculentus* corm. Some of these biochemical properties include saponfication value, unsaponifiable matter, peroxide, FFA value, and refractive index. The antifungal activity of the oil on some dermatophytes such as *Trichophyton, Microsporium, Candida, A. flavus*, and *Aspergillus niger* were also investigated.

Materials and Methods

Fresh *C. esculentus* corms (1000 g) were collected from Oyingbo market in Lagos State, Nigeria. They were packed in polythene bags and stored in a refrigerator prior to use. The percentage moisture content of the corm was determined at 103°C for 17 h as described by Agrawal (1980).

Extraction of oil

The method of extraction of oil of *C. esculentus* corm was adopted from the oil extraction methods of Egan *et al.* (1981). The corms were ground using a ceramic pestle and mortar before blending in an electric blender. An amount of 200 g of the ground corm was packed into the extraction thimble before covering with a small ball of cotton wool. The thimble was inserted in a quickfit plain body soxhlet extractor. Petroleum ether in the quantity of 200 mL (60–80°C) was poured in a 250 mL round-bottom flask of known weight,

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which was connected to the extractor, and refluxed on an electric thermal heater for 5 h. The ether was then collected in the plain body extractor and then separated from the flask that contained the oil. The flask containing the oil was then heated in an oven at 103°C for 30 min. It was cooled and weighed to get the final weight. The percentage oil content of the sample was calculated using the ratio of the amount of oil produced to the weight of sample used expressed as a percentage. The procedure was repeated until at least 250 mL of essential oil was extracted from the corm.

Biochemical properties of the essential oil of *C. esculentus* corm

The quantity of oil extracted from the corm was determined as a percentage of the oil extracted, expressed over the weight of the corm used, as described by Diamond and Denman (1973).

The method of Anonymous (1990) was used to determine the quality of oil extracted from the corms, the saponification value, unsaponfiable matter, peroxide value, and refractive index. The FFA value was determined according to the method of Egan *et al.* (1981).

Antifungal activity of the essential oil of *C. esculentus* corm

A modification of the paper disc diffusion method of Irobi and Daramola (1994) was used here. Spore or canidia suspension of 10^5–10^7 cells, were counted using a haemocytometer. About 10 mL sabouraud dextrose agar (SDA) were poured into Petri dishes and allowed to solidify. A micro-pipette was used to introduce 0.1 mL of the spore or conidia suspensions onto the agar plate; spreading was done with a spreading rod under sterile conditions. Sterilised paper discs (6 mm, Whatman No. AA2017006) were soaked in the *C. esculentus* oil for 6 h. The fungi used were collected from patients at the Department of Medical Microbiology Laboratory, University of Lagos Teaching Hospital, Idi-araba, Lagos. Four of these soaked discs were spread on a fungal inoculum seeded plate with the help of sterile forceps. There were two controls, the first contained the SDA and fungal inoculum but the discs were soaked in sterilised distilled water without the essential oil. The second control contained the SDA and fungal inoculum but the discs were soaked in an orthodox antibiotics, mycostatine solution (10 µg mL^{-1}). Three replicates were produced for each fungus per treatment. All the plates containing the discs were then incubated at 28–31°C. The zone of inhibition was measured after 48–72 h of incubation. The results were analysed using standard deviation analysis of variance (ANOVA, *F*-test), and Duncan's Multiple Range Test (Parker, 1979).

Effect of fungi on the biochemical property of artificially-infected *C. esculentus* oil

Eighty millilitres of the extracted oil was measured into eight test tubes, at 10 mL per test tube. Spore or conidia suspension of 10^5–10^7 cells, counted with haemocytometer were prepared from pure cultures of seven fungal species. The fungal species *A. jiauus, A. niger, Candida* sp., *Trichophyton mentagrophytes,* and *Microsporium gyseum* were isolated from patients at the Department of Medical Microbiology, University of Lagos Teaching Hospital, Idi-araba, Lagos. The other fungi used in this experiment, *A. wentii* and *Fusarium solani,* were isolated from melon seeds at the Botany research laboratory, University of Lagos, Nigeria. One millilitre of the spore or conidia suspension was added to seven of the test tubes, with one fungal species per test tube. The 8-mL test tube served as the control, and 1 mL of sterilised distilled water was added to it with no fungal inoculum. All the test tubes were shaken vigorously with an electric shaker for 1 h. Thereafter the test tubes were shaken everyday for 30 min. After 14 days of incubation, the biochemical property (FFA, peroxide value, refractive index at 40°C, saponification, and unsaponifiable matter) of the artificially infected oil in the test tubes was assessed as described earlier. The experiment was repeated twice. The results were analysed using standard deviation (SD), analysis of variance (ANOVA, *F*-test) and Duncan's Multiple Range Test (Parker, 1979).

Results

The water content of *C. esculentus* corm used in this study was 27.30 ± 0.40%, while the mean oil content (quantity of oil) of the corms was 15.44 ± 0.38%. The FFA content of the oil was 3.25 ± 0.27%; peroxide value was 5.26 ± 0.14 meq kg^{-1}; saponification value was 972.05 ± 26.81 mg KOH g^{-1}, and unsaponifiable matter was 61.70 ± 1.329 kg^{-1}.

The antifungal activity of the essential oil of *C. esculentus* corm on some dermatophytes is shown in Table 1. The oil showed antifungal activity against the five fungi tested, although the zone of inhibition was below 10 mm. The one exception was *T. mentagrophytes* which was 11.29 ± 0.24 mm. There was significant difference between the zone of inhibition of the oil by the fungi and their control. The antifungal activity of the antibiotic, mycostatine, was significantly higher than that of the oil.

Effect of fungi on the quality of artificially infected *C. esculentus* oil, after 14 days incubation, is shown in Table 2. There were significant changes by each of the fungi on the biochemical properties of the oil. The fungi increased the value of FFA, peroxide, and

Table 1 Antifungal activity of the essential oil by *Cyperus esculentus* corm

Sample	Zone of inhibition SD (mm)				
	Aspergillus niger	*A. flavus*	*Candida* sp.	*Microsporum gyseum*	*Trichophyton mentagrophytes*
Control	0.00±0.00 a[1]	0.00±0.00 a	0.00±0.00 a	0.00+0.00 a	0.00±0.00 a
Oil from *C. esculentus*	9.64±0.18 b	9.39±0.21 b	9.30±0.16 b	9.71±0.14 b	11.29±0.24 c
Mycostatine	15.30±0.12 d	15.20±0.40 d	16.70±0.17 e	17.40±0.13 e	15.50±0.10 d

[1]Zone of inhibition with different letters show significant difference ($P = 0.05$)

Table 2 Effect of fungi on the quality of *Cyperus esculentus* oil (after 14 days incubation)

Sample	Free fatty acid (%)	Peroxide value (meq kg^{-1})	Refractive Index at 40°C	Saponification value (mgkoH g^{-1})	Unsaponiliable matter (g kg^{-1})
Control (oil without any fungus)	3.38±0.60 a[1]	5.26±0.45 e	1.46±0.05 m	978.13±9.95 q	61.80±1.42 u
Oil with *Aspergillus flavus*	7.90±0.64 b	15.65±0.37 f	3.80±0.13 n	931.05±14.48 r	77.47±1.96 y
Oil with *A. niger*	7.80±0.44 b	14.34±0.93 g	3.73±0.18 n	927.40±12.76 r	75.33±1.46 y
Oil with *A. wentii*	7.40±0.81 c	13.21±0.59 f	3.47±0.11 p	915.36±11.82 s	71.38±1.27 x
Oil with Candida sp.	7.85±0.24 b	15.00±0.64 l	3.60±0.21 n	929.56±10.67 r	76.08±0.93 y
Oil with *Fusarium solani*	7.35±0.49 c	12.95±0.88 j	3.38±0.16 p	911.84±13.54 s	69.91±1.55 z
Oil with *Trichophyton mentagrophytes*	6.98±0.22 d	11.62±0.57 k	3.05±0.09 p	903.75±12.36 l	69.91±1.55 z
Oil with *Microsporcum gyseum*	6.90±0.50 d	10.32±0.76 l	3.02±0.12 p	902.00±11.05 t	65.87±1.54 w

[1]Treatment with different letters show significant difference ($P = 0.05$)

unsaponifiable matter of the oil, while the unsaponification value was decreased in comparison with the control. *Aspergillus flavus* had the highest value of the biochemical property among the fungi tested, while *M. gyseum* had the least value. The FFA value of the artificially infected oil was between 6% ± 0.50–7% ± 0.64%. Each of the fungus had varying changes on the biochemical property of the oil (Table 2).

Discussion

This study showed that the essential oil of *C. esculentus* corm is an edible oil. The qualitative properties of the oil fits the description of edible oils by Kirk (1991). The high saponification value of the oil indicates that it could be used as a base for soap manufacture. The peroxide value of the *C. esculentus* oil was 5.26 ± 0.14 meq kg^{-1}. Kirk (1991) explained that peroxide value below 10 meq kg^{-1} showed that the oil involved is a non-rancid oil. Also, the FFA content of the *C. esculentus* oil was 3.25 ± 0.27% and below the 5.00% FFA content recommended for non-rancid oil (Ekundayo and Idzi, 1990), implying that the oil is not rancid. However, the artificially-infected oils were rancid because the FFA value was between 6.90 ± 0.50 and 7.90 ± 0.64%, which is above the 5.00% recommended for non-rancid oils. These results indicated that the quality of oil can be affected by microbes during storage, since some of these fungi are also airborne (Adekunle, 2001). *Aspergillus flavus* caused the most devastating effect on the quality of the artificially-infected oil and support earlier work by Kuku (1979) who worked on palm kernel oil.

The essential oil from *C. esculentus* corm probably has a host-specific antifungal activity. It might not have a broad spectrum type of antifungal activity since it only inhibited the growth of *T. mentagrophytes* above the 10-mm mark recommended by Zygadlo and Grosso (1995). The work in this study complemented the report of other workers on essential oil of other plants, who claimed that essential oils could be fungitoxic, possessing a significant antifungal activity (Singh *et al.*, 1984; Owawunmi *et al.*, 1986; Begum *et al.*, 1993; Rana *et al.*, 1997). The commercial antibiotic, mycostatin, had a greater inhibition on the fungi than the extracted oil. This might be due to the fact that mycostatine is a purified antibiotic. If the oil's active ingredient is isolated and used, it might be more potent than the mycostatine at the same concentration.

The results of this study indicated the essential oil of *C. esculentus* to be edible, non-rancid, and saponifiable. The presence of fungi in the oil will change its biochemical composition making it rancid. Thus, proper storage and extraction under adequate sterile

conditions are recommended. These results indicate that the oil is fungitoxic, although it might be host-specific and that the essential oil from *C. esculentus* corm could be used to control plant fungal diseases under storage conditions.

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