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PHYTOCHEMICAL PROFILING AND ANTIFUNGAL POTENTIALS OF CASHEW NUTS (*ANACARDIUM OCCIDENTALE*) SHELL OIL

[1]Jesse Innocent Apameio, [1]Abalaka Moses Enemaduku, [1]Attah Friday and [2]Mela Mathias

[1]Department of Microbiology, Federal University of Technology, Minna, Nigeria
[2]Department of Microbiology, Federal University Wukari, Wukari, Nigeria
Correspondence author's Email address: jesseinnocent75@gmail.com
Telephone number: +234-7063090882

Abstract

The quest for antifungal substances due to fungi resistance to current synthetic antifungal drugs necessitates the discovery and development of herbal remedies from cashew nuts (*Anacadium occidentale* L.). Cashew has both medicinal and nutritional importance. Oil was extracted from the cashew nutshell using petroleum ether and *n*-hexane as a solvent with the aid of Soxhlex apparatus. The antifungal activity of these oil extracts was studied on *Trichophyton rubrum, Mucor pusillus, Alternaria* species*, Microsporum canis* and *Penicilium chrysogenum.* Some of these fungi were susceptible. From the Phytochemical analysis, it was found that the oil is rich in bioactive compounds, hence its antifungal activity. This oil might be used as a precursor in the development of novel antifungal substances to treat fungal infections.

Introduction

Cashew scientifically known as *Anacardium occidentale* L. is a member of the family Anacardiaceae. The origin of cashew is from Brazil and it was brought to Nigeria by the Portuguese voyage in the 16th century (Alia *et al.,* 2016: Hammed *et al.,* 2008). Cashew has numerous medicinal, nutritional and economical benefits. Its medicinal importance is because it is used in folk medicine for the treatment of diarrheal, skin infections, malaria and typhoid. Nutritionally it contains a high content of fats, proteins, carbohydrate, amino-acids and vitamins while its economic importance is attributed to its use as shad, the cashew apple, roasted kernel and nuts (Patrick *et al.,* 2011). Oil can be extracted from the shell of the nuts. Cashew nutshell oil (CNSO) have being previously reported by Kannan *et al.* (2009) to possess biological activities as mollusidal, larvicidal, pesticides (Garcia *et al.,* 2018) antibacterial and antifungal.

Fungal infections have emerged as one of the major causes of human disease, most importantly in immune-compromised patients (Shapiro *et al.,* 2011; Ghannoum and Rice, 1999). Diseases caused by fungi are usually superficial or on the skin can also lead to systemic infections as the disease incidence prolongs (Cannon et al. 2009; Brown et al. 2012). Among the different mycotic infections caused by these opportunistic fungi, candidiasis, are the most common caused by *Candida*, and with higher worldwide occurrence (Myers, 2006). Currently numerous antifungal drugs are ineffective due to the development of resistance. Therefore, the need to develop novel antifungal agents to fight against this mycosis.

Materials and Method

Collection and processing of the sample

Cashew nuts were collected from the plantation in Wukari, Taraba State, Nigeria. The nuts were air-dried for four weeks at room temperature, pulverised and kept for further analysis.

Extraction of CSNO

Two different solvents were used for extraction. This includes *n*-hexane and petroleum ether. Using a digital scale, 50 g of the pulverised sample measured into a thimble was placed in the extraction column of a Soxhlet apparatus containing 250 ml of solvent in the round bottom flask. Extraction was carried out using *n*-hexane and petroleum ether at a temperature of 35ºc and 45ºc respectively for three hours. The trapped extracts were concentrated using a rotary evaporator under a vacuum at temperature various boiling temperatures (Idah *et al.,* 2014; Yusup *et al.,* 2015).

Collection, isolation and identification of fungal sample

Samples were collected from patients with fungal skin infection, their skins were scrapped and the sample was inoculated on potatoes dextrose agar and incubated at 25ºc for 72 to 120 hours. The mixed culture obtain, the plate of the mixed culture was subculture to obtain a pure culture (Kanna *et al.,* 2009).

Macroscopic and microscopic identification

For macroscopic identification, the surface of the pure fungi isolates was observed with the aid of a magnifying lens.

For microscopic identification, a drop of 95 % ethanol was placed on a microscope slide. Using a sterile inoculating needle or fine forceps, a small portion of fungal growth was gently removed from midway between the colony centre and the edge. Take care that both the aerial and substrate mycelium was included in the specimen.

Using two dissecting needles, the fungus was gently teased so that it is thinly spread out in the mounting medium. When most of the ethanol has evaporated, a drop of lactophenol cotton blue was added and covered with a cover slip and the fungi were examined microscopically (Ochei and Kolhatkar, 2010).

Antifungal activity of CNSO

Fungal isolate was inoculated into potato dextrose broth and incubated for 18-24hours. The resulting turbidity was adjusted to 0.5 McFarland turbidity standard, using potato dextrose broth. A Swab stick was used to inoculate it on potato dextrose agar (PDA). Sterile cork borer was used to boreholes on the agar aseptically. A know concentration of the CNSO extract was dispensed into the hole. Plates were allowed to stay for 1hour before they were inverted and incubated at 25ºc for 24hours (Ochei and Kolhatkar, 2010). Dimethyl sulphoxide (DMSO) was used as negative control and nystatin was used as the positive control. The antifungal activity was assessed by the diameter of the zone of inhibition and results were recorded. This assay was performed in triplicate.

Qualitative phytochemicals analysis of CNSO

The extracts were tested for the presence of phytochemicals like alkaloids, carbohydrates, flavonoids, tannins, carbohydrate, glycosides, phenolics, steroids, terpenoids following standard methods previously described by Abalaka *et al.* (2009).

Data analysis

Results were expressed as the mean values ± standard error (S.E) of mean by measuring two independent replicates. Analysis of variance (ANOVA) using one-way was done and Duncan's test was performed to test the significant difference between means values obtained among the treatments at the 5% level of significance using Statistical Package for Social Science (SPSS) software (version 21, IBM SPSS). Differences were considered significant at $p<0.05$ (Das *et al.,* 2010).

Result

Percentage of CNSO yield

The result of the percentage yields of oil extracted from cashew nuts shell is shown in Table 1. Petroleum ether and *n*-hexane with different polarities were used for this extraction. Petroleum ether gave the highest percentage of oil yield (33.08%), as compared to n-hexane (30.31%). The CNSO is a dark brown oil, viscous, acre, and caustic.

Table 1 Result of percentage yield and volume of CNSO using *n*-hexane and petroleum ether

Solvent	Volume (ml)	% Oil Yield
n-Hexane	29.5	33.08
Petroleum ether	30.5	30.31

Results represent mean ± standard error of mean of duplicate determination.

Identification of fungal isolate

I. *Tricophyton rubrum*

Macroculture examination: moderately quick growing, mycelium radiating with elevated centre. The growth form was velvety to fluffy surface with central hat-shaped elevation. The upper side is white.

Microscopic examination: straight and septate hyphea. Microconidia are cone in shape.

II. *Microsporum canis*

Macroculture examination: relatively quick-growing, sometimes visible after 48 hours, fluffy to woolly. The upper side was white to yellow and the underside was yellow to orange.

Microscopy examination: The hyphae were ramified and septate, and usually straight.

III. *Mucor pusillus*

Microculture examination: quick growing, covering the agar surface in three days. Long-fibre and woolly network of hyphae which was white in colour.

Microscopic examination: the hyphae were thick and non-septate. Sporangiophores are departing laterally from the mycelium and the sporangia was filled with spore.

IV. *Penicilium* species

Microculture: grow very fast, velvety surface with many colour tones, green and blue-green.

Microscopy: the hyphae were septate with conidiophores rised vertically from the hyphae.

V. *Alternaria* species

Microculture: relatively slow growers with black to brown colony.

Microscopy: the hyphae were septate with numerous conidia (Figure 1).

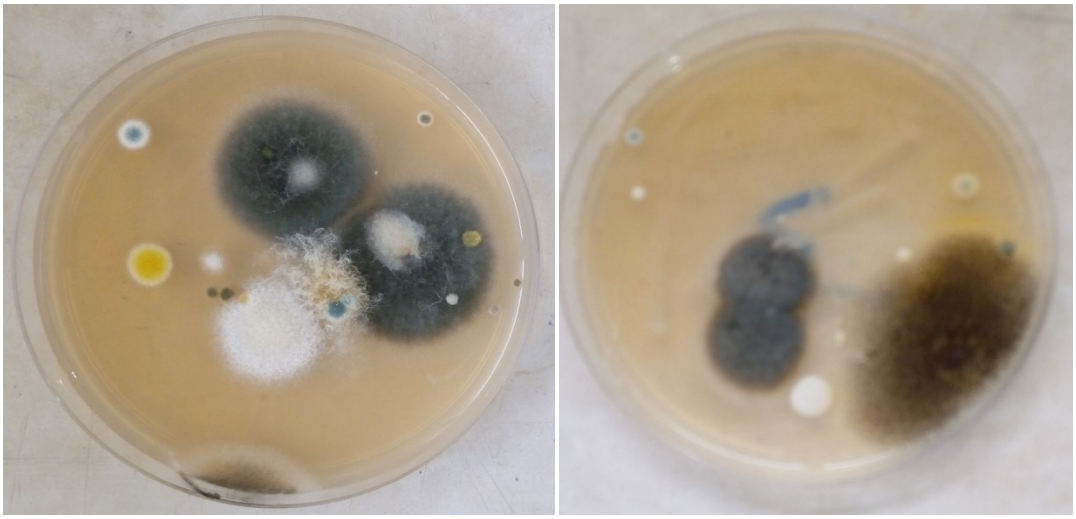

Figure 1. Colonies of fungi growing on culture media

Phytochemical analysis

Table 2 records the results of the phyto-constituents of CNSO using different solvent of extractions. Alkaloids, saponins, phenols, terpenoids, cardiac glycosides, steroids, phlobotannins were present in both the *n*-hexane and petroleum ether extract of the oil but flavonoids, tannins and reducing sugar were absent.

Table 2. Phytochemical composition of CNSO

	Solvents	
Phytochemical	**Hexane**	**Petroleum ether**
Saponin	+	+
Flavonoid	-	-
Tannins	-	-
Phenol	+	+
Reducing sugar	-	-
Glycoside	-	-
Alkaloid	+	+
Steroids	+	+
Phlobotannins	+	+
Terpenoid	+	+

Key

\+ = Present

\- = Absent

Antifungal susceptibility test

The results of antifungal susceptibility test of the oil extracts against standardized fungal isolates, *Trichophyton rubrum, Mucor pusillus, Alternaria* species*, Microsporum canis* and *Penicilium chrysogenum* using varying concentrations of 100 mg/ml, 200 mg/ml, 300 mg/ml and 400 mg/ml were shown in Table 3 below. The CNSO extracts were found to have activity on all the fungal pathogens showing varying zones of inhibition. *Trichophyton rubrum, Mucor pusillus* and *Penicillium chrysogenum* were found to be susceptible to *n*-hexane oil extract with the following zones of inhibitions 10-13 mm, 10 mm and 9 mm respectively while *Alternaria* species and *Microsporum canis* were resistant (Table 3). Table 3, revealed the following zones of inhibition: 8-9 mm, 9 mm and 9 mm for *Trichophyton rubrum, Alternaria* species and *Penicilium chrysogenum* respectively with petroleum ether CNSO. Dimethyl sulphur oxide (DMSO) showed no antifungal activity and so served as negative control. Nystatin was used as positive control which is known to express antifungal activity ranging from 16 to 20 mm for *Trichophyton rubrum, Mucor pusillus, Alternaria* species*, Microsporum canis* and *Penicilium chrysogenum* (Tables 3).

Table 3. Antifungal susceptibility test of CNSO against fungal pathogens

Solvent	***N*-Hexane**					**Petroleum Ether**				
CNSO/ Control (mg/ml)	*Trichophyton rubrum*	*Mucos pusillus*	*Alternaria species*	*Microsporum canis*	*Panicilium chrysogenum*	*Trichophyton rubrum*	*Mucos pusillus*	*Alternaria species*	*Microsporum canis*	*Panicilium chrysogenum*
100	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}
200	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}
300	10±1.00^{b}	0±0.00a	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	8±0.00^{b}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}
400	13±0.00^{c}	10±1.73^{b}	0±0.00^{a}	0±0.00^{a}	9±1.20^{b}	9±1.15^{b}	0±0.00^{a}	9±0.57^{b}	0±0.00^{a}	9±0.57^{b}
Nystatin	20±1.52^{d}	16±1.33^{c}	18±0.00^{b}	16±0.00^{b}	17±1.15^{c}	20±1.52^{c}	16±1.33^{b}	18±0.00^{c}	16±0.00^{b}	17±1.15^{c}
DMSO	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}

Results shows zone of inhibition size mean ± standard error of mean of duplicate determination. Mean values with the same superscript in the same column are not significantly different at $p<0.05$

Key: CNSO = Cashew nut shell oil; DMSO = Dimethyl sulphur oxide

Discussion

The total oil yields were calculated and expressed as percentage of sample weight (%, w/w). The yield of oil extract by petroleum ether gave 33.08% which was highest, followed by *n*-hexane with 30.31%. This might be due to the moisture content of the cashew nut shell which account for the oil yield from nuts. These suggest that petroleum ether is a good solvent to obtain large volume of CNSO from the shell of cashew nut. The results of the physical analysis showed that CNSO is dark brown in colour. Previous study by Idah *et al,* (2014) on CNSO, reported a 25% oil yield using *n*-hexane as solvent. In another study by Abitogun and Borokini (2009) that oil should constitutes about 47% of the total weight of the nut. The difference in oil yield could possibly be due to the method used in drying samples or the moisture content left in the nut shell before extraction (Table 1).

The two extracts of CNSO show antifungal activity at different concentration against *Trichophyton rubrum, Mucor pusillus, Alternaria* species*, Microsporum canis* and *Penicilium chrysogenum.* The antifungal activity might be as a result of phenols, saponins, flavonoids, terpenoids, tannins, alkaloids present in the oil extracts. Phenolic compounds are well known antifungal compounds that rapidly accumulate at the site of the infection in plants to resist microbial pathogens (Imelda, 2014; Matern and Kneusal, 1998). This result is at par with the findings of Amaral *et al*, (2016) and Jebapritha and Karpagam (2017). *Mucor pusillus* was found to be susceptible to *n*-hexane extract but resistant to petroleum ether, this could be that *n*-hexane was able to extract certain bioactive components from the cashew nut shell that petroleum ether could not, hence this bioactive components were responsible for the antifungal activity (Abitogun and Borokini, 2009).

Analysis of the phytochemical constituents of CNSO shows that the oil extracts were rich in phyto-constituents; saponins, terpenoids, phenols, alkaloids, steroids, phlobotannins and terpenoids were all present. This could be that this plant synthesizes these phyto-constituents from their normal metabolic processes for protection from excess sun light, herbivores and

defence against the attack of microorganisms. This result is in agreement with the findings of Jebapritha and Karpagam (2017) and Parasa *et al.,* (2011). It was observed that flavonoids, tannins, glycoside and reducing sugar were absent in both petroleum ether and *n*-hexane oil extract, this finding does not agree with a previous work reported by Jebapritha and Karpagam (2017). In this study methanol was used as solvent of extraction and flavonoids, tannins and reducing sugar were present, this could be as a result of its amphiphilic nature to dissolve both polar and non-polar bioactive components. These phyto-constituents have been known to exhibit medicinal physiological activities as reported by Oloninefa *et al.* (2018) and Nadia and Eleazar (2018).

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