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ANTIMICROBIAL ACTIVITY OF THEOBROMA CACAO POD HUSK EXTRACT ON ISOLATES OF TRYCHOPHYTON SPECIES FROM CASES OF *TINEA CAPITIS* AND RELATED KERATINIZED INFECTIONS

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ABSTRACT

This study evaluated the antimicrobial activity of Theobroma cacao pod husk extract on isolates of Trichopyton species; *T. mentagrophytes, T. tonsurans, T. rubrum* and *Microsporum canis* from *Tinea capitis* and keratinized infection in Ibadan. Fifty (50) clinical isolates of Trychophyton species in varied ratios were collected from dermatology unit of the University College Hospital Ibadan. The isolates were subcultured on to Saboraud Dextrose Agar medium fortified with 0.05mg of chloramphenicol and 0.2mg of cyclohexidime and then incubated aerobically at 25-30^{0}C for 72 hours and were biochemically differentiated with Urea broth. Phytochemical analysis of the extract and minimum inhibitory concentration (MIC's) were determined. The gender distribution ratios of the primary source of isolates were 70% males to 30% females. The N-hexane, ethyl acetate and methanol extracts of the CPH gave a yield of 1.29, 2.05 and 3.89 percent respectively. The minimum inhibitory concentration (MIC) of methanol ranged from 1.625 -3.125mg/mL, while the MIC's range of 12.5-50mg/mL and 0-12.5mg/mL were recorded for N-hexane and ethyl acetate extracts respectively. Tannins, saponins, flavonoids alkaloids were detected as bioactive compounds from the CPH extract investigated. The presence of bioactive compounds coupled with varied remarkable MIC's values recorded against the isolates in this study, is indicative of an antifungal potential of the cacao pod husk extract investigated.

KEYWORDS: **Antimicrobial effect, Cacao Pod Husk extract, Trychophyton species, *Tinea capitis*, Keratinized infection.**

INTRODUCTION

The increasing resistance to antibiotics has also resulted in search for organic molecules from plants with antimicrobial properties [2]. Bacteria have evolved numerous defenses against antimicrobial agents, and drug-resistant pathogens are on the rise. The resistance phenomena are multifaceted which could occur by multidrug resistance pumps (MDRs), membrane translocases that extrude structurally unrelated toxins from the cell. They tend to protect microbial cells from both synthetic and natural antimicrobials onslaught. Investigations of African medicinal plants for their antimicrobial activity rank highest among biological tests carried out on plants and their isolates [3]

Tinea capitis (ringworm of the scalp and hair) is a dermatophytic invasion and colonization of the hair and the scalp and are caused by dermatophytes in the genera of Trichophyton, Microsporum and Epidermophyton. It usually begins with hyphal invasion of the skin of the scalp, with subsequent

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spread down the keratinized wall of the hair follicle. The infection of the hair takes place just above the hair root. The hyphae grow down on the non-living portion of the hair and at the same rate as the hair grows upward. Infection produces dull gray, circular patches of alopecia, scaling and itching [4] Their survival and multiplication depends on host reaction to the metabolic products of this fungus. This contagious infection is commonly spread among children and play mates by body to body contact, sharing items that may not be clean, exchanging hair brushes, sleeping on the same mattress, exchanging pillows among the roommates and playing with sand or soiled particles. In prepubescent children, epidemic *Tinea capitis* is usually self-limiting. Zoophilic species may induce a severe combine inflammatory and hypersensitivity reaction called kerion- an exudative swelling covered with pustules. It is an exogenous infection that can be spread by direct contact with lesion or infected scales, hair etc. [5].

However, some predisposing factors such as overcrowding, environmental hygiene culture, health care, immigration, socioeconomic conditions, geographic location, climate (temperature, humidity, wind, etc.) have been incriminated as major factors for the variations in the distributions of the genus Trichophyton. (Trichopyton species; *T mentagrophytes, T. tonsurans, T. rubrum* and *Microsporum canis* which species can infect man and animals. Other spectrum of clinical signs include sparse hair coats or well-demarcated crusty lesions, with a chalky surface on the head (favus or ringworm) are its characteristic [6].

Cocoa has become an important ethno-medicinal plant since it has a unique chemical composition of more than 500 different compounds. Among their reported contributions to human health, there are the antioxidant, anti-inflammatory and antimicrobial activities of cacao pod husk (CPH) which contains dry matter, crude protein, ether extract, crude fibre, ash, acid detergent fibre, neutral detergent fibre and hemicellulose.

It is estimated that a ton of fresh cacao pod husk will produce about 16 kg ash after drying and burning. This ash contains about 40% potassium salts (Potash) mainly in the carbonate form. The soap made from CPH potash has been known for effective cleaning and also for maintaining healthy skin and hair. The tannin content is a potent antifungal. On the other hand, these cacao pod husk contains a range of biologically active compounds and therefore may represent a valuable source of isolation of substances of therapeutic value [7]. This study was carried out to determine the antimicrobial effect of Theobroma cacao pod husk extract on Trychophyton species, the etiologic agent of Tinea capitis conventionally known as ringworm and other related keratinized infection.

METHODS

Plant collection and authentication

Cocoa pod husk was collected from the processing control chamber of Cocoa Research Institute of Nigeria and was authenticated with a voucher number OOUPCGH 553 at the herbarium of the Department of Pharmacognosy of the Olabisi Onabanjo University.

Collection of isolates

A total of 50 clinical isolates of Trichophyton species from 35 males and 15 females were collected from dermatology unit of the University College Hospital in Ibadan.

Culture of isolates

Each clinical isolate was sub-cultured on to plates of Saboraud Dextrose Agar medium (Oxoid, Basingstoke, England) fortified with 0.05mg of chloramphenicol and 0.2mg of cyclohexidime. The plate culture was incubated aerobically at 28-30^{0}C for 72 hours and were biochemically differentiated with Urea broth, by which 16 isolates of T. mentagrophytes, 11 of T. tonsurans, 17 of T. rubrum and 6 of *Microsporum canis* were confirmed.

Microscopic Examination

Microscopic identification of mold isolates was performed by placing pieces of a colony from SDA to clean microscopic slide and staining with lactophenol -in -cotton blue. After placing a cover slip, each preparation was observed microscopically.

Macroscopic Examination

Macroscopic examination of cultures of Trichophytons colonies was carried out. Texture, rate of growth, topography, and pigmentation of the front and the reverse side of the culture were employed for the macroscopic identification.

Extraction of plant sample

A 10 g weight of dried and pulverized cocoa pod husk powder was soaked into 50mL organic solvents; viz, methanol, N- hexane and ethyl acetate separately for 24 hours in an orbital shaker

at room temperature. The extracts were filtered through the Whatman No.1 filter paper. The extracts were allowed to dry using rotary evaporator. The condensed extracts were stored in air-tight container at 4^0C.

Phytochemical Analysis

Phytochemical screening was performed to identify phytochemicals in methanol extracts of powdered cacao pod husk. The extract was subjected to phytochemical analysis using standard procedures [8].

Determination of Minimum Inhibitory Concentration

The Minimum Inhibitory Concentrations of the extract was determined by dissolving 0.5g of each dried powder extracts into 10 mL of (50%) methanol to attain a stock concentration of 50 mg/mL. Furthermore, 5 mL of the mixture was diluted serially in two-fold with 5mL of methanol (50%) resulting in concentrations of 25 mg/mL, 12.5 mg/mL, 6.125 mg/mL, 3.125 mg/mL and l.625 mg/mL respectively. The same procedure was repeated for each extract with ethyl acetate and n-hexane extract respectively. Two milliliter (2mL) of each dilution of extract was mixed with 18 mL of Saboraud Dextrose Agar medium, poured into Petri-dishes and allowed to set. Each plate was inoculated with 1:100 dilution of the broth culture of each of the test organism and incubated at 28 - 30^0C for 72 hours. The plates were then examined for the presence of growth after the incubation period. The least concentration that gives no visible growth of the test organism was taken as MIC's of the extract.

RESULTS

Of the 50 isolates of Trichophyton spp collected, 16 were *Trichophyton mentagrophytes*, 11 *Trychophyton tonsurans*, 17 *Trichophyton rubrum* and *6 Microsporum canis*. The gender distribution ratio of the primary source of isolates was (70%) males and (30 %) females (Table 1). The n-hexane, ethyl acetate and methanol extracts gave a yield of 1.29, 2.05 and 3.89 percent respectively (Table 2).

The phytochemical screening of the cocoa pod husk extract indicated the presence of saponins, alkaloids and tannins while anthraquinones were absent (Table 3). The minimum inhibitory concentration of methanol extract ranged from 1.625 -3.125 mg/mL, while the MIC's range of 12.5-50 mg/mL and MIC's ranged between 0.813-12.5 mg/mL were recorded for N-hexane and ethyl acetate extracts respectively (Table 4).

DISCUSSION

The gender distribution ratio of the primary source of samples was (70%) males and (30%) females. The prevalence and the distribution patterns of the isolates of dermatophytes with respect to gender were higher among males than females. This agrees with reported that the management of *Tinea capitis* in childhood scalp infection in females is less due to steroid-mediated inhibition of dermatophyte growth by progesterone and other similar compounds, while males may be predisposed to scalp infection due to prepubertal factors such as level of fungistatic fatty acid [9]. Various conflicting views exist regarding the gender predominance of *Tinea capitis* which may be attributed to feminine hair styling, shaving of the scalp, plaiting, and the use of hair oils with some degree of antimicrobial components which may prevent hair parasites. However, the precise role of such practices remains a subject of study [10].

The percentage yield of methanol extract was higher (3.89%) than ethyl acetate extract (2.05%) and n-hexane extract (1.29%). The higher yield could be attributed to selective reaction of the composition of the powdered husk to chemicals of different polarity. This agrees with earlier studies on the antimicrobial potentials of the pod husk phytochemicals [10][11]. Alkaloids, saponins and tannins are secondary bioactive metabolites that could account for the antifungal activity of the cocoa pod husk extract against the dermatophytes [12].

The variation in the MIC's obtained in this study could be due to polarity of the solvent used [13], the inherent factors within the isolates [14] and the genetic variations of the isolates [15]. The resistance of some isolates to ethyl acetate could be attributed to intrinsic or extrinsic factor within the host [16] and application of hair treatment ointments that organism could develop resistance to [17].

Domestic animals such as cats, dogs, chickens, cattle, and horses can serves as reservoir of zoophilic dermatophytes, most often seen on exposed body sites. The prevalence of anthropophilic dermatophytes may reflect important risk factors in the spread of Tinea capitis and other related keratinized. An etiological shift with regard to the predominant causative agents of Tinea capitis has been reported in different parts of the world including the United States, Europe, and

Table 1: Age and gender distribution of the primary source of the fungal isolates

Age(years)	Number examined	*Tricophyton metagrophyte*	*Trichophyton tonsurans*	*Trichophyton rubrum*	*Microsporum canis*
3-5	15	3	3	3	1
6-8	12	5	2	4	0
9-11	9	3	4	4	1
12-14	8	3	2	3	2
15-17	6	2	2	2	1
Male	35	12	8	10	5
Female	15	4	3	7	1
Total	50	16	11	17	6

Table 2: Extraction yield values of cocoa pod husk extract

	N-Hexane	Ethyl acetate	Methanol
Weight of Dried CPH(g)	200	200	200
Weight of extract(g)	2.57	4.10	7.78
Percentage Yield (%)	1.29	2.05	3.89

Table 3: Phytochemical constituents of cocoa pod husk extract

S/n	Test	Result
1	Anthraquinones	-
2	Flavonoids	+
3	Saponins	+
4	Alkaloids	+
5.	Tannins	+

Keys: Present: + absent : -

Table 4: Minimum Inhibitory concentration of cocoa pod husk crude extracts on test organisms.

Test organisms	MIC (mg/mL) Methanol extract	N-Hexane extract	Ethyl acetate extract)	Methanol (50 %)	Ketoconazole (1 %)
Tm1	1.625	12.5	12.5	-	0.50
Tm2	3.125	12.5	0.813	-	0.125
Tm3	1.625	12.5	12.5	-	0.125
Tm4	3.125	12.5	0.813	-	1.00
Tm5	3.125	12.5	0.813	-	1.00
Tm6	3.125	12.5	0.813	-	0.50
Tm7	1.625	12.5	3.125	-	0.25
Tm8	1.625	12.5	3.125	-	0.125
Tm9	3.125 '	12.5	3.125	-	0.125
Tm10	3.125	12.5	1. 625	-	0.50
Tm11	1.625	12.5	3.125	-	0.125
Tm12	3.125	12.5	1.625	-	0.50
Tm13	3.125	12.5	0.813	-	0.50
Tm14	3.125	25.0	0.813	-	1.00
Tm15	1.625	12.5	0.813	-	0.50
Tm16	1.625	12.5	3.125	-	0.25
TtI7	1.625	12.5	1.625	-	0.50
Tt18	1.625	12.5	3.125	-	0.125
TtI9	1.625	50	1.625	-	0.25
Tt20	1.625	12.5	6.25	-	0.25
Tt.2I	1.625	12.5	3.125	-	0.25
Tt22	1.625	50	3.125	-	0.25
Tt23	1.625	12.5	3.125	-	1.25
Tt24	1.625	12.5	0.813	-	0.25
Tt25	1.625	12.5	1.625	-	0.125
Tt26	1.625	12.5	1.625	-	0.125
Tt27	1.625	12.5	0.813	-	0.25
Tr28	1.625	50	3,125	-	0.25
Tr29	1.625	12.5	3.125	-	0.125
Tr30	1.625	12.5	0.813	-	0.50
Tr31	1.625	12.5	12.5	-	0.125
Tr32	3.125	12.5	0.813	-	0.125
Tr33	1.625	12.5	12.5	-	1.00
Tr34	3.125	12.5	0.813	-	1.00
Tr35	3.125	12.5	0.813	-	0.50
Tr36	1.625	12.5	12.5	-	0.25
Tr37	3.125	12.5	0.813	-	0.125
Tr38	1.625	12.5	12.5	-	0.125
Tr39	3.125	12.5	0.813	-	0.50
Tr40	3.125	12.5	0.813	-	0.125
Tr41	3.125	12.5	0.813	-	0.50
Tr42	1.625	12.5	3.125	-	0.50
Tr43	1.625	12.5	3.125	-	1.00
Tr44	3.125 '	12.3	3.125	-	0.50
Mc45	3.125	12.5	1.625	-	0.25
Mc46	1.625	12.5	3.125	-	0.50
Mc47	1.625	12.5	12.5	-	0.125
Mc48	3.125	12.5	0.813	-	0.25
Mc49	1.625	12.5	12.5	-	0.25
Mc50	3.125	12.5	0.813	-	0.25

Keys: Tm:*Trychophyton mentagrophyte*, Tt:*Trychophyton tonsurans* Tr: *Trychophyton rubrum* Mc:*Microsporum canis*

parts of Nigeria. This is attributed to factors that aid the spread of dermatophyte; by migrants, change in animal husbandry practices, climate change, and evolution of new genotypes [18].

Although *in vitro* activity cannot be directly translated into clinical use, the MIC exhibited by the extract, coupled with the presence of bioactive compounds make the extract a potential candidate for the treatment of dermatophytic infections.

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