Antifungal Activities and Phytochemical Constituents of *Nicotiana tabacum* Leaf Extracts on Selected Dermatophytes.

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Abstract: Extracts *of Nicotiana tabacum* were investigated for phytochemical constituents and antifungal activities against certain dermatophytes: *Epidermophyton floccosum , Microsporum canis* and *Trichophyton rubrum* . The alcohol extract was made using 95% ethanol and 95% methanol as extracting solvent in the ratio of 2:1. The hot water extract was made by boiling sample in distilled water. The phytochemical analysis revealed the presence of carbohydrates, glycosides, fats and oil, saponin, and tannins. Alkaloids were in appreciable quantities while cyanogenicglycosides were absent. The crude extracts were acidic and exhibited antifungal activity against all the test organisms. The minimum inhibitory concentrations of the extracts on the organisms varied between 7.8 and 62.5mg/ml. The treatments were significantly different at p=0. 05. The mode of action of the extracts varied with the test organisms. This study revealed antifungal effect of leaf extract of *Nicotiana tabacum* on *Epidermophyton floccosum , Microsporum canis* and *Trichophyton rubrum*

Keywords:

1. Introduction

Naturally occurring bioactive compounds with antimicrobial properties are gaining considerable attention as attractive eco friendly alternative to synthetic antimicrobial agents *Nicotianatabacum* (tobacco) leaf belongs to the family *Solanceae*. It is an amphibioid between two species of *Nicotiana* namely, *N. sylvestris*, *A. otophora* (Wikipedia 2011). It is found only on cultivation. When the leaves are dried and ground into fine particles, it is taken as snuff, which is a very common form of tobacco taken in West tropical Africa. Researchers now use extracts of plants to analyse the pharmaceutical activities of hidden secondary metabolites in them (*Duengsri et al* 2012).

Dermatophytic fungal infections (dermatomycosis, tinea, ring worm) are one of the most common infectious diseases in the world and are among the most commonly diagnosed skin diseases in Africa (Ribbon, 1988;Nweze, 2010).)

This is even more true in tropical climates. *Trichophyton rubrum , T. mentagrophytes* complex or *Trichophyton* spp. are the main etiologic agents of dermatophytoses. Mateja, (2016) defined Dermatophytes as a group of closely related filamentous fungi that have the capacity to invade the keratinized tissue of skin, hair and nails in humans and animals. The invasion can cause diseases of skin, hair, and nails. There are three genera of dermatophytes: *Epidermophyton, Micosporum* and *Trichophyton*. Infections involving these organisms are transmitted by contact, from animal to man, from soil to man and from man to man. (Amphawan *et al*, 1995).

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The prevalence of these infections have been on the increase in recent times. This has been attributed to ageing of the population; changes in immune response occasioned by age increased numbers of immune suppressed patients, HIV infected persons and those who have diabetes or other chronic diseases.

A lot of work have been done on antibacterial agents compared to antifungal agents. This has led to more available antibacterial drugs and in contrast has led to a rise not just in prices of the few antimycotics available, but also to the development of resistance by pathogenic fungi to available drugs. The number of therapeutic options for the treatment of fungal infections are quite limited compared with those available for bacterial infections. Only one new class of antifungal drugs has been developedin the last 30 years (Terry and Damian, 2014).

Many plants like *Borreriaverticillata, Acalyphatorta, Ceibapentandra, Zingiberofficinale. Cymbopogoncitratus, Loranthusbengwelensis* etc. have been investigated for antifungal activities. Unfortunately, most of these researches never went beyond preliminary screening stages. (Nwachukwu *et al* 2008).

This work aims at going beyond preliminary tests to drug development and incorporation in the Nigerian pharmacopaedia.

2. Materials and methods.

Nicotinanatabacum, leaf was obtained from a farm at Soba, near Zaria in Kaduna State of Nigeria and identified at the Federal College of Forestry, Jos, Nigeria.

The tabacco leaves were dried in an oven at 40^0C for 72hours, after which it was ground into tiny particles by means of a sterilized mortar and pestle. The extraction was by means of Soxhlet apparatus. Two extracts were prepared. One by use of hot water and the

other by using 95% ethanol apd 95% (v/v)methanol in the ratio 2:1 as extracting solvents. Twenty grams of the ground leaves were extracted using 200ml of extracting solvents for 24hours. The resulting solution was filtered using what man filter No.1. The filtrate was concentrated by evaporating gently using a rotary evaporator. This was stored in an airtight container and refrigerated until required for use. It was labeled 'stock solution.'' Reconstitution and dilutions of the stock solutions was by use of sterile saline for the aqueous extracts and Dimethyl Sulphoxide (DMSO) made by Aldrich U.K for athe alcohol extracts to avoid sterile filtrations. These were subsequently filtered through 0.22µm sterile filter Millipore as in the methods of Ginvyanet *et al*, 2017 and Yash *et al*, 2017).

The phytochemical analytical methods used were as described by AOAC, (1980), Iwu (1982), Emeruwa (1982),Onwuliri (1996) and Yash (2017). The criteria used for identification of the dermatophytes were as those described by Rebel and Taplin (1970), Campbell and Stewart (1980) and Mateja (2016). The identified test organisms used were *Epidermorphytonfloccosum, Microsporum canis and Trichophyton rubrum*. The microorganisms were used as activity growing cultures. They were isolated from school children in Kaura Namoda Local Government Area of Zamfara State Nigeria after permission was obtained from the Ministry of Education Zamfara State. Cultures are deposited at Microbiology Laboratory 1, Federal university of Technology Owerri Nigeria, they are designated EF1, TR1, and TR1 for *Epidermorphyton floccosum, Microsporum canis and Trichophyton rubrum* respectively.

The media used for identification was Sabouraud Dextrose Agar (SDA) and Potatoe Dextrose Agar (PDA). The antifungal studies was on SDA only. All media used were fortified with yeast extract and chloramphenicol at 0.5mg/l to discourage growth of bacteria. The determination of antifungal activity of the extracts was by standard cup plate method. The test organisms were standardized in a broth to contain 103 spores/ml. (Conidia counts were performed by means of a Neubauer chamber). One ml of this was used to flood the surface of SDA in a culture plate and excess culture on the surface of the agar poured off. The agar was dried at 37°c for 15mins. Six wells were bored with NO.4 sterile corkborer with diameter of 8mm. These wells were filled with various concentrations of the doubly diluted extracts. This was to allow for diffusion of the extracts into the agar. The plates were incubated at 26°C for 10 days. Wells containing 1%DMSO (v/v) and wells without fungal spores in each plate served as growth and sterility controls respectively (Biasi-Garbin, 2016).

Minimum Inhibitory Concentrations (MIC) of the extracts were determined for the test organisms. This was achieved by microdilution method. Sample concentration range was prepared from the stock solution bt two fold dilution in the sterile diluents with concentrations ranging from 3.9 tq 250 mg/ml was tested. MIC was taken as the lowest concentration of the extract that inhibited visible growth of the test organisms (Ginovyan, 2017; Mostafa, 2017; and Elisha, 2017). Minimum Fungicidal Concentrations (MFC) of the extracts on the test organisms was also determined. Sample of micro-organisms from plates used in the MIC assays which did not show growth after the period of incubation was diluted 1.4 with fresh media and 50m1 amounts sub- cultured on fresh medium as described by Rotimi *et al.*(1988) and Sales *et al.* (2016). The minimum fungicidal concentration (MFC) was regarded as the lowest concentration of the extracts that did not permit any fungi colony growth after 10davs of incubation. The results were subjected to statistical analysis, using the Analysis of Variance(ANOVA).

3. Results.

The colour of the alcohol extract was observed to be yellowish green and had a pH of 5.3. The colour of the hot water extract on the other hand was brown and showed a pH of 5.90. The percentage extractions achieved for the alcohol and hot water extracts are 6,8% and 4.4% respectively. The phytochemical analysis of the extracts revealed the presence of carbohydrates, fats and oil, saponins, alkaloid and tanins. Cyanogenic glycosides were absent. (Table 1.)

The results of the antifungal assay showed that all the test organisms are sensitive to both the alcohol and hot water extracts of tobacco leaf. However, the effects varied with the concentration of the extracts and test organisms. The greatest inhibitory effect was shown by the hot water extract on *Microsporiumcanis* followed by the alcohol extract on the same organism. The least action was observed for the alcohol extract on *Epidermophyton floccosum*. The treatments are significantly different at P= 0.05.

The minimum inhibitory values of the extracts on the test organisms ranged between 7.8mg/mi to 62.5mg/ml. The highest values of 62.5/ml was observed for both extracts on *E. floccosum*. The lowest values of 7.8mg/ml was observed for water extract on *M. canis*. (Table 3).

The minimum fungicidal concentration values of the extracts on the test organisms are much higher than the MIC values of the extracts on the test organisms are much higher than the MIC values. No fungicidal activity was recorded for any of the extracts on *E.floccosum*. The levels of 62.5mg/ml and 31mg/mi were recorded for alcohol and hot water extracts respectively. No fungicidal action was observed for *M. canins and T. rubrum*, with alcohol extracts whereas the hot water extract was 62.5mg/ml. (Table3)

3. Discussion/Conclusion

The antifungal activities of the alcohol and water extracts of *N. tabacum* compared favourably with each other. This shows that the inhibitory component is extractable and soluble in both water and alcohol. The

phytochemical analysis revealed the presence of appreciable amounts of alkaloids, trace of *tanins and saponins*. This is in line with the findings of Yash (2017). These compounds are known to have biological activities. Onwurili (1996) and Yash (2017) observed that nicotine, an alkaloid is the most biologically active component of tobacco. However, nicotine might not necessarily be responsible for the fungicidal action. Davidson and Branen (1993) and Fiori *et al.* (2013) found that tanins have profound antimicrobial activities and that amongst tannins; tannic acid has the highest inhibitory activity. It has also been shown recently that saponins have very useful antifungal properties and that they are active against *Candida Albicans* and other fungi (Nwachukwu *et al.*, 2008). The inhibitory action of these extracts might also be as a result of the presence of phytoalexins. They are a group of compounds that help protect plants from diseases. The high MIC values recorded in this study could be greatly reduced by identifying and purifying the component responsible for the desirable action of the extracts. The antifungal activities of these extracts observed in this study raises hope of their potential as a source of medicine against a wide range of dermatophytosis .Work is however continuing on in vivo studies, identification and purification of the bioactive component responsible for this inhibitory action.

Table 1: Phytochemical Analysis of *N. tabacum leaf* extracts

a.	Carbohydrates		d.	Saponins	
	Fhelings test :	+		Froth test	+
				Haemolysis	+
b.	Alkaloids				
	Meyers test:	++	e.	Tanins	
	Wagners test:	++		Rhubard test:	+
	Dragendoffs test:	++			
			f.	Steroids and terpmoids	
c.	Cyanogenic glycoside				
	Sodium picrate test:	-		Solkowskis test	+

Keys: += present in tracequantity
++= present in appreciable quantity
- = absent

Table 2.Antifungal susceptibility assay of the Alcohol and Hot water extracts. Of*N.tabacum on E. floccosum, M. canis and T. rubrum*

	DilutonConc (mg/ml)	Zones of inhibition in mm					
		E.floccosum		*M.canis*		*T.rubrum*	
Stock	**Standard**	**AE**	**HWE**	**AE**	**HWE**	**AE**	**HWE**
1	250	9.1	9.3	10.0	12.0	8.2	9.3
2	125	8.5	8.7	8.7	11.7	7.0	6.0
3	62.5	6.0	6.0	8.1	11.0	6.0	5.0
4	31.3	5.0	5.2	7.2	8.0	5.0	2.0
5	15.6	-	-	7.2	6.0	2.0	-
6	7.8	-	-	5.0	5.0	-	-
7	3.9	-	-	-	4	-	-
Control 3		-	-	-	-	-	-
		3.0	-	3.2	-	3.0	-

(Mean of 4 Assays).

Key: AE = Alcohol Extract. HWE = Hot water extract.
- = No inhibitory activity observed.

Table 3:MinimumInhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of *N. tabacum* extracts on the test organisms (in mg/ml)

E.floccosum			*M. canis*		*T.rubrum*	
	AE	HWE	AE	HWE	AE	HWE
MIC	62.5	62.5	15.6	7.8	31.3	62.5
MFC	-	-	62.5	31.3	-	62.5

Key: AE = Alcohol Extract -= fungistatic activity up to undiluted extract
HWE = Hot water Extract.

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