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PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTY OF *DATURA STRAMONIUM* ON SOME ORAL PATHOGENIC MICROORGANISMS

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ABSTRACT
Datura stramonium was examined for its antimicrobial activity on some oral pathogens using methanol, chloroform, acetone and water as extractants. The organisms used were *Streptococcus mutans* and *Candida albicans*. The phytochemical properties of the plant revealed the presence of glycosides, alkaloids, steroids, flavonoids and tannins. The crude extract exerted different inhibitory effects on *Streptococcus mutans* and *Candida albicans* at 2000 µg/ml, 3000 µg/ml, 4000µg/ml and 5000µg/ml. The minimum bactericidal concentrations (MBC) were 60mg/ml and 100mg/ml and fungicidal concentration (MFC) was 60mg/ml. The minimum inhibitory concentrations (MIC) of the extracts were 40mg/ml, 60mg/ml and 80mg/ml respectively. The antimicrobial activity of the plant showed that *Streptococcus mutans* was susceptible to the leaf extract of acetone at 4000µg/ml and 5000µg/ml while chloroform were susceptible to both the leaf and seed extracts in all the concentrations used. *Candida albicans* was susceptible to methanol seeds and aqueous leaf extract in all concentrations while the chloroform leaf extract was also active at 3000µg/ml to 5000µg/ml for the leaf extract. This signifies that the methanol seed and aqueous leaf extract are highly active against *Candida albicans*. The thin layer chromatography (TLC) results confirmed the presence of fractions of different R_f values ranging from 0.38-0.65. It can be deduced that the leaf and seed extract of *Datura stramonium* possess more than one active component. The fractionated chromatograph of all the extracts of both leaf and seed of *Datura stramonium* had no antimicrobial activity on the test organisms.

KEY WORDS: *Datura stramonium*, phytochemical, thin layer chromatography, *Candida albicans*, *Streptococcus mutant.*

INTRODUCTION

Man has made use of various parts of plants in the treatment and prevention of various ailments (Shagal *et al*., 2012). Traditional and folklore medicines play important roles in health services around the globe. About three quarter of the world's population rely on plants for health care (Premanathan *et al*., 2000; Gabhe *et al*., 2006). A World Health Organization survey indicated that about 70-80% of the world's population rely on non-conventional medicine, mainly of herbal source, in their primary healthcare (WHO, 2007). Most of these herbal remedies have stood the test of time, particularly for the treatment of allergic, metabolic and cardiovascular diseases. (Igoli *et al*., 2005). The interest in the scientific investigation of medicinal plants from Nigeria is based on the claims of their effective use for the treatment of many diseases. Therefore research into the effects of these local medicinal plants is expected to enhance their use against diseases caused by these microorganisms (Sofowora, 1993, Johnson *et al.*, 2011). The use of medicinal herbs in the treatment and prevention of diseases is attracting scientists' attention worldwide. This is corroborated by World Health Organization in its quest to bring primary health care to the populace (Falodun *et al*., 2006, Ameen *et al*., 2010). In Nigeria, nearly all plants are associated with some medicinal values. The use of plants especially in traditional medicine is currently well recognized and accepted in Nigerian health care practice (Hassan and Kamba, 2010). Plants usually contain phytochemicals which are active substances technically referred to as drugs, and over the years these drugs have been exploited as traditional medicine for the treatment of various ailments afflicting man (Shagal *et al*., 2012)

The aims and objectives of the study are to test the therapeutic effectiveness of the leaf and seed extract of *Datura stramonium* against *Candida albicans* and *Streptococcus mutans*, determine the phytochemical and antimicrobial activities of *Datura stramonium*, and evaluate the Minimum Inhibitory Concentration (MIC), Bactericidal Concentration (MBC) and Fungicidal Concentration (MFC) of the extracts. Examine purified fractions of the crude extracts on Thin Layer Chromatography (TLC) as well as the activity of the fractions on *Candida albicans* and *Streptococcus mutans.*

MATERIALS & METHODS

Collection and identification of Plant

Fresh leaves and seeds of *Datura stramonium* were collected from Bosso Secondary school quarters, Minna, Niger State. Nigeria. The plant materials were identified by Prof. N.I.S. Ezenwa of the Crop production department in School of Agriculture and Agricultural sciences, Federal University of Technology, Gidan Kwano Minna, Niger State. The leaves were washed and air dried over a period of 3 weeks. The dried samples were pounded and milled into fine powder with the aid of sterile mortar and

pestle. The fine powder were collected into a sterile aluminum foil and kept in a cool dry place till further use.

Test organism

The microorganism used for this study, bacterium: (*Streptococcus mutans*), fungi: (*Candida albicans*) were collected from the stock culture in the laboratory of Microbiology Department, Federal University of Technology, Minna, Niger State, Nigeria. The bacteria isolates were sub cultured into Nutrient Agar and incubated at 37 ^{o} C for 18 hours while the fungi isolate was sub cultured into freshly prepared Sabouraud Dextrose Agar (SDA) slant and incubated at room temperature and All were stored in the refrigerator for subsequent use.

EXTRACTION OF PLANT MATERIALS

Dried leaves and seeds (90g) were weighed and extracted using 400ml of distilled water methanol, acetone and chloroform using reflux extraction technique. The extracts were placed on water bath to evaporate. The dried extracts were stored in sterile universal bottle (Silver *et al.,* 1997).

Phytochemical screening of plant extracts

The phytochemical screening of the extract was carried out according to the method described by Trease and Evans (1999).

Test for Alkaloid: One milliliter of 1% HCl was added to three 3mls of the extract in a test tube. The mixture was then heated for 20 minutes, cooled and filtered. Two drops of Wagner's reagent to 1ml of the extract was added. A creamy precipitate indicated the presence of alkaloids.

Test for Tannins: One milliliter of freshly prepared 10% KOH was added to 1ml of the extract. A dirty precipitate showed the presence of tannin.

Test for Cardiac Glycoside: Zero point five grams of the powdered samples was placed in a test tube and 2.5mls of water was added to the sample. A prepared moist sodium picrate was suspended in the neck of the test tube by means of a cork. The test tube was placed in a water bath for one hour. A brick red color on the paper indicated the presence of glycosides.

Test for Saponnin: Three drops of distilled water was added to two drops of each extract and vigorously shaken with the test tube for some seconds. A positive result was indicated by the presence of frothing or bubbling.

Test for Steroids (Salkowski test): Five drops of concentrated H_2SO_4 were added to 1ml of the extract in a test tube. Red coloration was observed which indicated the presence of steroids.

Test for Anthraquinone: Zero point five grams of *Datura stramonium* was placed in a flask and 10ml of chloroform added and shaken for 5minutes. The extract was then filtered. A bright pink color in the upper aqueous layer indicated the presence of free anthraquinone.

Test for flavonoids: One milliliter of each extract was added with NaOH solution. The appearance of a yellow coloration which then disappeared on addition of HCl indicated the presence of flavonoids.

Test for phenolics: Two drops of 5% $FeCl_3$ were added to1ml of the extract. The presence of greenish precipitate indicated the presence of phenols.

Standardization of microorganism: A loop full of test organism was inoculated in 5ml of sterile Nutrient Broth and incubated for 18 hours. Zero point two of 18 hours culture of the organism was dispensed into 20mls of sterile Nutrient Broth and incubated for 3-5 hours to standardize the culture to 10^6 Cfu/ml. A loop full of the standardized cultures was used for the antimicrobial activity (Babayi *et al*., 2004).

Screening of Extract for Antimicrobial Activity (Agar Ditch Method)

Sterile Nutrient agar plates were prepared and seeded with standardized bacterial inoculums using sterile cotton swabs. Sterile cork borer (diameter 4mm) was used to bore wells on each plate. Pasteur pipette was then used to transfer different concentrations of each plant extract on each labeled bored well, while the fourth well was for Procaine Penicillin and Histatane as control for bacteria and fungi respectively. The plates were left for 15 minutes to allowed for maximum diffusion into the medium. The bacteria plates were incubated at 37^{o}C for 24 hour and fungi for 48 hours at room temperature. The diameter zones of inhibition around the wells were measured and recorded. Antimicrobial and anti-fungi activity were expressed as the average diameter of the zones of inhibition.

Determination of minimum Inhibitory Concentration (MIC): The broth dilution method was employed, 9ml of nutrient broth and potato dextrose broth was dispensed into 10 test tubes each, and these were sterilized at 121^{o}C for 15mins and allowed to cool at room temperature. The tubes were labeled 1- 10. One milliliter (1ml) from the re-constituted extract was introduced into the test tubes.1ml of the standardized inoculum was also added to the broth, the test tubes were then incubated for 24hrs at 37^{o}Cfor bacteria and 48hrs at ±27^{o}Cfor fungi. Turbidity was observed. The tube with the visible growth was taken and recorded as the MIC.

Determination of minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

Samples of organism were taken from the Nutrient agar plates and Sabouraud dextrose agar plate that showed no visible growth after 37^{o}C for 24 hours and sub cultured into freshly prepared sterile Nutrient agar and Sabouraud dextrose agar. The least concentration that did not produce growth after 24 hours and 48 hours was regarded as the MBC and MFC respectively.

Thin Layer Chromatography (TLC)

Mini thin layer chromatographic plate was prepared using microscopic slide. The slides were cleaned with acetone and washed with hot water to remove all stains, dirt's and oil marks. 2g of silica gel (Merek AR60) was mixed with 4mls of distilled water and ground in a mortar until it began to thicken. The slurry was then poured on the slide and spread evenly with a glass spreader and allowed to set for 5 minutes. It was dried at 110^{o}C in an oven for at least 15 minute to activate the paste and allowed to cool by exposing the plates to the atmosphere for 30 minutes. Excessive silica gel was carefully removed from the slide using a razor blade. The preparation of the macro-plate involved the same procedure using 20 x 10cm glass plates. 25g of silica gel was mixed with sterile distilled water. Chromatographic separation of the methanol, acetone, chloroform and aqueous extracts of *Datura stramonium* leaf and seed on the prepared macro plates involved the use of a solution of dried extract which was made by

dissolving 1g of extract 3mls of the solvent. A capillary tube was used to make a concentrated band of the solution on the TLC plate about 2cm from the base of the plate. The mobile phase used was a mixture of Chloroform and methanol in a ratio of 5:1 and 5:2 which was put in a chromatography glass tank. The tank was closed and allowed to stand for about ten minutes so that the tank becomes saturated with solvent. The plates were inserted into the tank with the origin spot towards the bottom of the tank. The glass tank was covered tightly and the solvent allowed to ascend until it gets close to the top. The plates were removed and dried in the oven. Bands of separated fractions were detected under UV light. Distance moved by the solvent and that moved by the extract were measured. (Oyeleke *et al*, 2008).

Determination of antimicrobial activity of fractions (TLC) The resultant bands containing the activity of the reconstituted extract was determined against the test organisms using standard cultures. Each extract (0.01g) was weighed and dissolved in 5ml of their respective solvents to give a concentration of 2mg/ml. Sterile agar medium was prepared in sterile petri dishes and the standardized culture inoculated. Wells were made and filled with the respective extracts and control antibiotics. The plates were incubated at 37^{o}C for 24 hours after which zones of inhibition were observed and measured.

RESULTS

TABLE 1: Phytochemical screening of *Datura stramonium*

Test	Extract							
	Acetone		Methanol		Aqueous		Chloroform	
	leaves	seed	leaves	seed	leaves	seed	leaves	seed
Saponin	-	-	-	-	-	-	-	-
Glycoside	++	+	-	+	+	-	-	-
Steroids	-	+	-	+	-	-	+	-
Alkaloids	+	+	+	++	-	-	-	-
Tannins	-	-	+	-	+	-	-	-
Flavonoids	++	-	-	+	-	-	+	-
Anthraquinone	-	-	-	-	-	-	-	-

Keys: ++ = highly present; + = moderately present; - = absent.

Table 1 revealed the phytochemical properties of the leaves and seeds of *Datura stramonium* which showed the presence of glycoside, steroids, alkaloids, tannins and flavonoids.

TABLE 2: Antimicrobial activity of crude leaves extract of *Datura stramonium* showing the zone of inhibition (2000-5000 µg/ml)

Organisms		Concentration (µg/ml)			
		2000	3000	40000	5000
Streptococcus mutans	C	16	18	25	27
	M	-	-	-	-
	A	-	-	17	19
	CH	15	21	22	23
	W	8	9	22	22
Candida albicans	C	25	26	23	25
	M	-	-	-	-
	A	-	-	-	-
	CH	-	27	28	29
	W	23	25	25	26

Keys: - = No activity; C = control (procaine for bacteria and fushin for fungi)
M = methanol, A = acetone, CH = chloroform, W = water

Table 2 revealed the activities of the leaves crude extracts against the test organisms. The bacterium (*Streptococcus mutans*) was resistant to methanol extract while the fungus (*Candida albicans*) was resistant to methanol and acetone extract.

TABLE 3: Antimicrobial activity of crude seed extract of *Datura stramonium* showing the zone of inhibition (2000-5000 µg/ml)

Organisms		Concentration (µg/ml)			
		2000	3000	40000	5000
Streptococcus mutans	C	16	18	25	27
	M	-	-	-	20
	A	-	-	17	-
	CH	-	-	-	-
	W	-	10	-	-
Candida albicans	C	25	26	23	25
	M	16	25	27	29
	A	-	-	-	-
	CH	-	-	-	-
	W	-	-	-	-

Keys: - = No activity; C = control (procaine for bacteria and fushin for fungi
M = methanol, A = acetone, CH = chloroform, W = water

TABLE 3 revealed the activities of the leaves crude extracts against the test organisms. The bacterium (*Streptococcus mutants*) was resistant to methanol extract while the fungus (*Candida albicans*) was resistant to methanol and acetone extract.

TABLE 4: Minimum inhibitory concentration (MIC) of the crude leaves extract *Datura stramonium.* MIC values in (mg/ml)

Organisms	Extras			
	Methanol mg/ml	Acetone mg/ml	chloroform mg/ml	Aqueous mg/ml
Streptococcus mutans	-	80	40	40
Candida albicans	-	-	60	40

Keys: - = turbidity

Table 4 revealed the minimum inhibitory concentration (MIC) of the leaves extract values of the susceptible test organisms for acetone, chloroform and water. *Streptococcus mutans* had the highest MIC.

TABLE 5: Minimum inhibitory concentration (MIC) of the crude seed extracts *Datura stramonium. MIC* values in (mg/ml)

Organisms	Extras			
	Methanol mg/ml	Acetone mg/ml	chloroform mg/ml	Aqueous mg/ml
Streptococcus mutans	80	-	-	-
Candida albicans	40	-	-	40

Key: - = turbidity

Table 5 revealed the minimum inhibitory concentration (MIC) of the seed extract values of the susceptible test organisms for methanol and water. *Streptococcus mutans* had the highest MIC.

TABLE 6: Minimum bacteriocidal concentration (MBC) of the leaves and seed extracts of *Datura stramonium.* MBC values in (mg/ml)

Organisms	Extras			
	Methanol mg/ml	Acetone mg/ml	chloroform mg/ml	Aqueous mg/ml
Streptococcus mutans	-	-	-	60
Candida albicans	100	-	-	-

Key: - = turbidity

Table 6 revealed the minimum bactericidal concentration (MBC) of the leaves and seed crude extracts of *Datura stramonium*. The MBC shows 60mg/ml (aqueous) and 100mg/ml (methanol) respectively.

TABLE 7: Minimum fungicidal concentration (MFC) of the leaves and seed extracts *Datura stramonium. MIC* values in (mg/ml).

Organisms	Extras			
	Methanol mg/ml	Acetone mg/ml	chloroform mg/ml	Aqueous mg/ml
Streptococcus mutans	-	-	60	-
Candida albicans	-	-	-	-

Keys: - = Growth

Table 7 revealed the minimum fungicidal concentration (MFC) of the leaves and seed extracts of *Datura stramonium*. The MFC shows 60mg/ml (chloroform) but do not show MFC for the seed extract.

TABLE 8: Thin layer chromatography (TLC) of *Datura stramonium* leaf and seed extract. $CHCl_3$: MeOH 5:1 and 5:2

Extracts	visible spot	UV	R_f value	Rf value
Acetone (leaf)	green	white	0.54	0.38
Methanol (leaf)	-	white	0.64	0.59
Acetone (seed)	-	white	0.56	_
Chloroform (leaf)	-	white	0.45	0.05
Aqueous (leaf)	-	white	0.49	0.63
Methanol (seed)	-	white	0.50	0.69
Aqueous (seed)	-	-	-	
Chloroform (seed)	-	-	-	

Keys: - = No spot

Table 8 revealed the visible spots and the retardation factor (R_f) values of different component in the fractions of *Datura stramonium* (leaf and seed) chloroform ($CHCl_3$) methanol (MeOH) ratio of 5:1 and 5:2 solvent systems.

DISCUSSION

The phytochemical and antimicrobial activities of the leaves and seeds extract of *Datura stramonium* revealed the presence of alkaloids, glycoside, flavonoids and tannins. This is at variance with the reports of Aderotimi and Adeyemo (2006) and Shagal *et al.* (2012) in which glycosides, tannins and flavonoids were absent. This could be due to the extractants used and the parts of the plant worked on (Table 1). The crude methanol leaves extract showed no activity against *Streptococcus mutans and Candida albicans,* while the crude acetone extract showed activity at 4000 µg/ml and 5000 µg/ml concentrations against *Streptococcus mutans.* However, this concentration was inactive for *Candida albicans.* The crude Chloroform and aqueous leaves extract showed activity for both *Streptococcus mutans* and *Candida albicans*. The crude methanol seed extract showed activity against *Candida albicans* at 2000µg/ml, 3000µg/ml, 4000µg/ml and 5000µg/ml concentrations (Tables 2 and 3). The minimum inhibitory concentration (MIC) ranged from 40mg/ml to 80mg/ml (Tables 4 and 5) while the minimum bactericidal concentration (MBC) was 60mg/ml and 100mg/ml (Table 6). The minimum fungicidal concentration (MFC) was 60mg/ml (Table 7). The thin layer chromatography (TLC) results confirmed the presence of fractions of different R_f values (Table 8 and 9), it can be deduced that the leaves and seed extracts of *Datura stramonium* possess more than one active component. The fractionated chromatography of the methanol, chloroform, acetone and aqueous extracts of both the leaves and seed of *Datura stramonium* reveal the presence of alkaloid which had no antimicrobial activity on the test organisms. This was in agreement with Sofowora (1993) who reported that the effectiveness of activity of a medicinal plant does not depend on one fraction but rather a combination of fractions.

CONCLUSION

This study revealed the presence of secondary metabolites in both seeds and leaves of *Datura stramonium*. It has further confirmed that the leaves may be used for the treatment of infections caused by these pathogens. The results give credence to the traditional use of the plant in the treatment of diseases caused by *Candida albican* and *Streptococcus mutant*.

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