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Original Article

INHIBITION OF *CANDIDA ALBICANS* AND *STREPTOCOCCUS MUTANS* WITH DATURA LEAF AND SEED EXTRACTS

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ABSTRACT

Objectives: Existing antimicrobial compounds are of a limited effectiveness. Therefore, the antimicrobial activity of *Datura stramonium* extracts was evaluated against two important human pathogens; *Candida albicans* and *Streptococcus mutans*.

Methods: In this study, *Datura stramonium* was collected from Al Baha area and its leaves and seeds were extracted with different organic solvent. *Datura stramonium* extracts were tested for the inhibition of *Candida albicans* and *Streptococcus mutans* using Agar Ditch method. Also, the minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) were evaluated

Results: The minimum bactericidal concentrations (MBC) of Datura were 65 mg/ml in case of leave water extract and 100 mg/ml of methanol seed extrac when tested against *S. m.u.tans*. However, MFC was 65mg/ml in the case of chloroform and water leave extracts and 100 mg/ml with methanol seed extract, when tested against *C. albicans*. The minimum inhibitory concentration (MIC) of the plant leaf extracts was 80 mg/ml of acetone, 45 mg/ml of both chloroform and water extracts when tested on *S. mutans*. Chloroform and water leave extracts were inhibitory to *C. albicans* with MIC: 45 and 60 mg/ml respectively. Furthermore,*Candida albicans* was susceptible to methanol seed and aqueous leaf extract at 45 mg/ml.

Conclusion: In the future, these extracts might be tested as amendments to teeth past to avoid its decay by *S. mutans* or be used as an ointment against candidiasis infection. Where, it has proven inhibitory efficacy on tested microorganisms inhibition.

Keywords: *Datura stramonium,* Phytochemical, Thin layer chromatography, *Candida albican*, *Streptococcus mutant*.

INTRODUCTION

In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains with a multidrug-resistant [1]. The non-availability and the high cost of new antibiotics generation with a limited effectiveness span have resulted in an increase in morbidity and mortality [2]. Dental caries is a disease caused by specific type of bacteria live in the human mouth. These bacteria produce acid that destroys tooth enamel and results in cavities on its surface. Among the oral micro flora pathogens are almost exclusively streptococci which is the main causative agent for dental caries in developing countries people. The principal causal agent of dental caries in the group of Streptococcal species, is *Streptococcus mutans* and *Streptococcus mites* of human dental caries [3]. On the other hand, The therapy of deep fungal infections, particularly those caused by opportunistic pathogens, such as *Candida albicans*, remains a difficult medical problem as mentioned by many investigators. Actually, antifungal treatments need lifelong therapy, because of its toxicity and high cost of their production.

The use of plant extracts in the treatment and prevention of diseases is attracting scientist's attention all over the world. This is corroborated by the World Health Organization in its quest to bring primary health care to the populace [4, 5]. Plants usually contain phytochemical active substances which are 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 [6]. The type of solvents and methods of preparation affect antimicrobial activity of plants [7]. The acceptance of traditional medicines as an alternative form of health care has led researchers to investigate the antimicrobial activity of medicinal plants [8]. Many investigators have listed that the crushed datura leaves are used to relieve pain, due to its antoxidants, antimicrobial and phytochemical contents. *Datura stramonium* Linn (Solanaceae) grows as a wasteland weed. Very small amount of work has been done on the antibacterial activity of this medicinal plant, it need further study for verification of its activity against disease causing microorganisms.

Datura plant is an important medicinal plant as it is a well known source of different phytochemicals (secondary metabolites), and it is distributed throughout most of the part of the world.

The objective of the study was to test the therapeutic effectiveness of the leave and seed extracts of our local plant variety of *Datura stramonium* to evaluate their effect against *Candida albicans* and *Streptococcus mutans* growth inhibition.

MATERIALS AND METHODS

Collection and identification of plant

Plant fresh leaves and seeds were collected from the Al - Baha city, Saudi Arabia. The plant materials were identified as *Datura stramonium* Mill. The leaves were washed and air dried over a period of 3 weeks. The dried sample was pounded and milled into a fine powder with the aid of sterile mortar and pestle. The fine powder was collected into a sterile aluminum foil and kept in a cool dry place till further use.

Test organism

The microorganism used for this study was bacterium: *Streptococcus mutans* that was obtained from Microbial culture collection (Botany Dept. Faculty of Science, Tanta University, Egypt) and *Candida albicans* SC5314 was gifted from VA Medical Center, Yale University, Infectious disease dept. to Yehia Mahmoud by Vernon Kalb. Bacterial isolates were subcultured into nutrient agar and incubated at 37^{o}C for 18 hours while the *Candia albicans* SC5314 isolate was subcultured into freshly prepared sabouraud dextrose agar slant and incubated at room temperature. All were stored in the refrigerator for subsequent use.

Extraction of plant material

Forty five grams of dried leaves and seeds were weighed and extracted using 200 ml* of distilled water (universal solvent), methanol, acetone and chloroform (polar solvents) using reflux extraction technique. The extracts were placed in the water bath for

all the solvent to evaporate. The dried extracts were stored in a sterile universal bottle and stored [9].

Mocro organism standardization

A loop full of test organism was inoculated in five milliliters (5 ml*) of sterile Nutrient Broth and incubated for 18 hours. Exactly zero point two (0.2 ml*) of 18 hours culture of the organism was dispensed into twenty milliliter (20 ml*) of sterile Nutrient Broth and incubated for 3-5 hours to standardize the culture to 10^6cfu/ml. A loop full of the standardized culture was used for the antimicrobial activity [10].

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 4 mm*) was used to bore wells on each plate. Sterile Pasteur pipette was then used to transfer different concentrations of each plant extract on each labeled well, while the fourth well was for procaine penicillin and histatin as standard positive control for bacteria and fungi respectively. The plates were left for 15 minutes to allow for maximum diffusion into the medium. The bacteria and fungi plates were incubated at 37^{o}C for 24 hours and 48 hours respectively. Diameter zones of inhibition around the wells were measured and recorded, along with tested samples; positives antibiotic controls were used. Procaine penicillin was used in case of bacterium and Nystatin for Candida at 2,3, 4 and 5 mg/ml. Antimicrobial activity was expressed as the average diameter of the inhibition zones of three replicates. .

Determination of minimum inhibitory concentration (MIC)

MIC was evaluated according to Collins *et al.* [11]. T.h.e broth dilution method was employed, nine milliliter (9 ml*) of nutrient broth and potato dextrose broth was dispensed into several numbers of test tubes, and this was sterilized at 121^{o}C for 15 min*s. This was allowed to cool to room temperature and was labeled 1- 6. One milliliter (1 ml*) from the re-constituted extracts was introduced into the test tubes. One milliliter (1 ml*) of the standardized inoculums was also added to the broth, the test tubes were incubated for 24 hrs* for bacteria and 48 hrs* for fungi and turbidity was observed. The tube with the visible growth was taken and recorded as the MIC and the mean has been calculated and recorded for three experiments.

Determination of minimum bactericidal concentration (MBC) and minimum candidicidal 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 and the mean has been calculated and recorded for three experiments.

RESULTS

As we mentioned, there is an urgent need for new antimicrobial compounds that are more potent. *Streptococcus mutans* is one of the main agents responsible for human tooth corrosion, also *C. a.l.bicans* causes human candidiasis. *Datura stramonium* leaves and seeds were extracted with different chemical solvents and its antimicrobial activities were tested against both tested organisms. table (1 and 2) indicates that the activities of the leave crude extracts against the tested organisms.

Streptococcus mutans was resistant to methanol extract while *Candida albicans* was resistant to acetone extracts of Datura leaves and seeds. However, Datura leaves chloroform extract exhibited a good inhibition vales nearly equal to that of standard antibiotics used in case of *S. m.u.tans* and *C. albicans*. table (3) reveals that the minimum inhibitory concentration (MIC) of the leave extracts values of the test organisms for acetone, chloroform and water. *Streptococcus mutans* had the highest MIC value of 45 mg/ml of crude Datura chloroform and water leaves extracts.

Table 1: Antimicrobial activity of crude leaf extract (2-5 mg/ml)of *Datura stramonium* showing the inhibition zone (mm).

Organisms	Concentration (mg/ml)				
	Plant extract type	**2**	**3**	**4**	**5**
Streptococcus mutans	Procaine Penicillin *	15	17	26	28
	Methanol	0.0	0.0	0.0	0.0
	Acetone	0.0	0.0	17	19
	Chloroform	15	20	22	24
	Water	7	10	23	23
Candida albicans Sc5314	Nystatin	25	26	25	25
	Methanol	0.0	0.0	0.0	0.0
	Acetone	0.0	0.0	0.0	0.0
	Chloroform	0.0	27	28	29
	Water	23	25	25	26

*C= Positive control (Procaine Penicillin for bacteria and Nystatin for Candida)

●Inhibition zone (mm) is the mean of 3 replicates

0.0 = No Growth

Table 2: Antimicrobial activity of crude seed extract of *Datura stramonium* showing the inhibition zone (mm).

Organisms	Concentration (mg/ml)				
	Plant extract type	**2**	**3**	**4**	**5**
Streptococcus mutans	Procaine Penicillin*	15	19	26	28
	Methanol	0.0	0.0	0.0	20
	Acetone	0.0	0.0	17	0.0
	Chloroform	0.0	10	0.0	0.0
	Water	0.0	26	0.0	0.0
Candida albicans SC5314	Nystatin*	25	25	24	25
	Methanol	16	0.0	27	29
	Acetone	0.0	0.0	0.0	0.0
	Chloroform	0.0	0.0	0.0	0.0
	Water	0.0	0.0	0.0	0.0

*C= Positive control (Procaine Penicillin for bacteria and Nystatin for Candida)

●Inhibition zone (mm) is the mean of 3 replicates

0.0 = No Growth

Table 3: Minimum inhibitory concentration (MIC) value (mg/ml) of Datura stramonium crude leave extracts

Organisms	MIC● value of crude leaf Extract (mg/ml)			
	Methanol	Acetone	Chloroform	Water
Streptococcus mutans	0.0	80	45	45
Candida albicans SC5314	0.0	0.0	60	45

●MIC is the mean of three experiments

Table (4) reveals the minimum inhibitory concentration (MIC) of the seed extracts values of the susceptible test organisms for methanol and water. *Candida albicans* had the highest MICvalue of 40 mg/ml of crude seed methanol and water extracts. However, the MIC value of crude seed extract in case of *S. mutans* was 85 mg/ml when testing methanol seed extract.

Table 4: Minimum inhibitory concentration (MIC) values in (mg/ml) of the *Datura stramonium* crude seed extracts

Organisms	MIC● value of crude seed Extract (mg/ml)			
	Methanol	Acetone	Chloroform	Water
Streptococcus mutans	85	0.0	0.0	0.0
Candida albicans SC5314	40	0.0	0.0	40

0.0 = No Growth ●MIC is the mean of three experiments

Table 5: Minimum bacteriocidal concentration (MBC) values in (mg/ml) **of the leave and seed extracts of *Datura stramonium***

Organisms	Extract type	MBC● of Extracts (mg/ml)			
		Methanol	Acetone	Chloroform	Water
Streptococcus mutans	Leaves	0.0	0.0	0.0	65
	Seed	100	0.0	0.0	0.0

0.0 = No Growth ●MBC is the mean of three experiments

Table 6: Minimum fungicidal concentration values in (mg/ml) (MFC) of the leave and seed extracts *Datura stramonium*

Organisms	Extract type	MFC● value of Extracts (mg/ml)			
		Methanol	Acetone	Chloroform	Water
Candida albicans SC5314	Leave	0.0	0.0	65	65
	Seed	100	0.0	0.0	0.0

0.0 = No Growth ●MFC is the mean of three experiments

Table (5) shows the minimum bactericidal concentration (MBC) of the leave and seed crude extracts of *Datura stramonium*. The MBC shows 60mg/ml (aqueous) in case of leaves extract and 100mg/ml with methanol extract for *S. mutans*. table (6) reveals the minimum fungicidal concentration (MFC) against *C. albicans* of the leave and seed extracts of *Datura stramonium*. The MFC shows 65 mg/ml (chloroform and water) but do not show MFC for the seed extract. Whereas, MFC value was 100 mg/ml with seed methanol extract (*C. a.l.bicans*).

DISCUSSION

Plants constitute an important source of drugs in modern medicine. The antimicrobial activities of the leave and seed extract of Datura stramonium have been evaluated against *S. m.u.tans* and *C. albicans*. The crude methanol leave extract shows no activity against *S. mutans* and *C. a.l.bicans*, while the crude acetone extracts shows activity at 4 mg/ml and 5 mg/ml concentrations against *Streptococcus mutans*. However this concentration was inactive for Candida albicans. The crude Chloroform and aqueous leave extract shows activity for both *S. mutans* and *C. albicans*. The crude methanol seed extract shows activity against Candida albicans at concentrations of (2-5 mg/ml) (Tables 1 and 2). The minimum inhibitory concentration (MIC) ranges from 40 mg/ml to 85 mg/ml (Tables 3 and 4) while the minimum bactericidal concentration (MBC) was 65 mg/ml and 100 mg/ml. The minimum fungicidal concentration (MFC) was 60 and 100 mg/ml. The methanol, chloroform, acetone and aqueous extracts of both the leave and seed of *Datura stramonium* reveals the presence of alkaloid which had antimicrobial activity on the tested organisms, and this is almost in agreement with [11, 12].

However, phytochemical and the antimicrobial activities of the leave and seed extract of Datura stramonium reveals the absence of glycoside, tannin and flavonoid [12, 6]. The S. mutans glucosyltransferase synthesize extracellular polysaccharides, mainly hydrophobic glycan from sucrose, colonize the tooth surface and initiate plaque formations [13].

We propose that Datura extract might have inhibitors that might block the activity for glucosyltransferase (data to be presented elsewhere). Further study should be done to identify and purify the active ingredients of *D. stramonium* leaves and seeds. These extracts might be added in teeth past to prevent its decay by *S. mutans* or added to ointments from prevent or cure Candidiasis attached to human skin.

CONCLUSION

This study reveals the presence of secondary metabolites in both seeds and leaves of Datura stramonium which might be responsible for the inhibition of the growth of both *C. albicans* and *S. mutans*. It has further confirmed that the leave may be used for the treatment of infections caused by tested pathogens. The results give credence to the traditional use of the plant in the treatment of diseases caused by *Candida albican* and *Streptococcus mutant*.

CONFLICT OF INTEREST

The authors report that there is no conflict of interest.

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