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ANTIBACTERIAL AND PHYTOCHEMICAL INVESTIGATION OF *DATURA STRAMONIUM* L LEAF EXTRACTS AGAINST CLINICAL WOUND SAMPLES

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ABSTRACT

Context: *Datura stramonium* Linn is one of the most common Indian medicinal plants commonly Used as medicine to cure various diseases. This study determined the presence of virulence factors in the organism, correlates it with the multi-drug resistance (MDR) and antibacterial, phytochemical activities of the leaf of *Datura stramonium* against wound causing pathogens. **Aims:** The aim of the following study is evaluate the antibacterial, phytochemical activity of the different extracts of *D. stramonium* leaf sample against selected bacterial isolates. **Subjects and Methods:** Purulent materials from different wound infections samples received were processed as per standard microbiological procedures. Virulence factors of biofilm were studied. Antibiotic sensitivity test was performed by standard Kirby Bauer disc diffusion technique. Antimicrobial activity of various extracts of plant leaves was investigated by agar disc and well-diffusion method against wound causing bacterial isolates. The phytochemical activities of leaf extract were done as per Clinical and Laboratory Standard Institute Guidelines. **Results:** Each collected wound samples showed one or more bacterial isolate, totally 78.9% of samples exhibited 43 isolates. Among the 18 antibacterial agents, the most of the antibacterial agents had highly resistance activity and some agents had highly sensitive activity against the isolates. All isolates were having the ability to produce biofilm, among them 58% of isolates were strong Biofilm producers. The antibacterial studies, the acetone extract of *Datura stramonium* presented the highest anti-*S. aureus* activity and was effective

against all bacterial strains tested. Phytochemical screening of the plant revealed the presence of alkaloids, tripenoid, steroids, flavonoid, triterpenes, phenolic compounds and tannins. **Conclusions:** The general results observed consider the plant as natural source of phytoconstitution quality for antimicrobial effectiveness.

KEYWORDS: Wound, Clinical bacteria, extracts, antibacterial, photochemical, *Datura stramonium.*

INTRODUCTION

A wound is a breach in the skin and the exposure of subcutaneous tissue following loss of skin integrity provides a moist, warm, and nutritive environment that is conducive to microbial colonization and proliferation.[1] Infection in a wound delays healing and may cause wound breakdown, herniation of the wound and complete wound dehiscence.[2] In spite of technological advances that have been made in surgery and wound management, wound infection has been regarded as the most common nosocomial infection especially in patients undergoing surgery.[3] It is an important cause of illness resulting in a prolongation of hospital stay; increased trauma care, treatment costs, and general wound management practices become more resource demanding.[1] The severity of the complications depends largely on the infecting pathogen and on the site of infection[4, 5] and in general, a wound can be considered infected if purulent material is observed without the confirmation of a positive culture. The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and to a greater incidence of infections caused by Polymicrobial flora.

Management of wounds is a complicated and expensive program and research on drugs that increase wound healing is a developing area in modern biomedical sciences. Several drugs obtained from plant sources are known to increase the healing of different types of wounds. Though some of these drugs have been screened for evaluation of their wound healing activity, the potential of many of the traditional used herbal agents remains unexplored. In few cases active chemical constituents were identified.[6]

The medicinal plant *D.stramonium* is often used as an analgesic plant in folklore medicine in the "Old world".[7] *Datura stramonium* is an erect annual herb forming a bush up to 1.5 m tall. The leaves are soft, irregularly undulate and toothed. The flowers are trumpet-shaped, white to creamy or violet and 6 to 9 cm long.[8] The alkaloid content of *D. stramonium* has

been emphasized by the phytochemical investigators dealing with the biochemical composition of various parts of the plant.[9] Atropine, hyoscyamine and scopolamine (hyoscine) are the tropane alkaloids of all species of the genus *Datura* and their concentrations showed variations depending on species and on the part of the plant.[10] Duke (1992a) presented data on the analysis the concentration of total alkaloids in leaves and flowers of *D. metel* (Hindu datura), *D. stramonium* (Jimson weed) and *D. innoxia* (Thorn apple). The emphasis on tropane alkaloids is due to their importance in pharmaceutical industry. Proteins, fats, fatty acids, reducing sugars, oxalates, nitrates and tannin are among the chemical entities that have been described in the plant.[12,13] Chlorogenic acid, an antihistaminic, an allantoin, an immunostimulant[14], Lectin agglutinin, a glycoprotein[15, 16], gamma amino-butyric acid, a hypotensive and neuro-inhibitor[17], are within the list of the active ingredients present in *D. stramonium*.

In allopathic drugs separate wound healing antibacterial compounds were used where as harbours may considered as single source of cells. Having known the traditional use of this plant as an wound healing agent. The present study attempt was made to screen antibacterial activity of *D. stramonium* against the wound. The pathogen isolated from the directly evident wound (pus) samples. To understand the effect of this plant preliminary phytochemical study Was done along with antimicrobial activity against would pathogens.

SUBJECTS AND METHODS

Sample population: This study was carried out from wound patients of hospitals in and around Namakkal district during the study period (July 2011 to March 2012). Patients (n=100) both male and female included in this study were residents of Namakkal, TN.

***Collection of wound pus samples*:** A total of 100 pus swabs were obtained from wound sites before the wound was cleaned using 70% alcohol. The specimen was collected on sterile cotton swab without contaminating them with skin commensals. Different types of wound samples were collected namely accident wound, post-operation sepsis, skin infection, abscesses and burn wound. All samples were collected from hospitals in Namakkal and properly labeled indicating the source and age of patients. The samples were transported soon to the laboratory after being obtained. In the laboratory, the specimens were registered and swabs were cultured on various media and incubated at 37ºC for 24 h.[18]

Isolation and identification of wound bacterial isolates: Culture plates of Eosin methylene blue agar, MacConkey agar, Nutrient agar, Cetrimide agar and Mannitol salt agar (Hi Media, India) were used. The swab sticks used for the collection of the samples were streaked directly on the labeled agar plates and incubated at 37°C for 24 h. After incubation, cultures were examined for significant growth. Subcultures were then made into plates of nutrient agar and incubated for another 24 h. The primary identification of the bacterial isolates was made based on colonial appearance and pigmentation. Biochemical tests were performed to identify the isolates. Biochemical tests applied were standard catalase test, citrate utilization, coagulase, oxidase, methyl red, Voges-Proskauer, indole production, motility, carbohydrate fermentation test using glucose, sucrose, maltose and lactose. Characterization and identification of the isolates was done using the methods of Cowan and Steel (1985), Cheesbrough (2004), Mathur *et al.* (2006) and Senthilkumar *et al.* (2012).

Antibiotic sensitivity assay: The standard Kirby-Bauer disk diffusion method was used to determine the antibacterial profiles of the isolates. The nutrient broth was prepared and sterilized at 121°C at 15 min and inoculated the isolates then incubated at 37°C for 24 hrs. After incubation period the broth culture were swabbed into surface of the Mueller-Hinton agar plates and antibiotic discs were placed, then Plates were incubated at 37°C for 18 to 20 h. The zone of inhibition and resistance was measured, recorded and interpreted according to the recommendation of the disc manufacture's standard chart.[19]

Collection and Drying of plant materials: Apparently healthy leaves of *Datura stramonium* were collected from Ladapuram village, Perambalur district, Tamil Nadu, India. The leaves of *D. stramonium* were washed thoroughly three times with water and once with distilled water. The plant leaf were dried for 3 weeks and then ground to powder with a mechanical grinder.

Extraction of Powdered Plant Material: The powders obtained were extracted separately with various organic solventsat room temperature (25±2°C). The resulting crude extracts were filtered and evaporated in shaker water bath maintained at 55-65°C. The obtained semi-dried crude extracts were contained in plastic containers and labeled appropriately as AE (Aqueous Extract), EE (Ethanol Extract) ME (Methanol Extract), CE (Chloroform Extract), PE (Petroleum Ether) and AE (Acetone Extract).

Antimicrobial Activity of the Crude Extract: The antibacterial screening of the various extracts was assessed against clinical bacterial strains isolated from various wounds infection. The bacterial isolates include: *Streptococcus sp*, *Pseudomonas aeruginosa*, *Escherichia coli*, *S. aureus*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Enterococcus faecalis*. Nutrient agar and nutrient broth were used for the sub culturing of the bacterial isolates. Mueller-Hinton agar (Himedia) was used for the bacterial sensitivity screening. The antibacterial screening of the crude extracts were evaluated by agar well diffusion.[20] The crude extracts were reconstituted in 5% V/V aqueous dimethylsulphoxide (DMSO) at concentration of 20mg/ml. The inocula of the test bacterial isolates were prepared from 24h broth culture. The absorbance was read at 530nm and adjusted with sterile distilled water to match that of 0.5Mc Farland standard solution. From the prepared bacterial suspension, other dilutions were prepared to give a final concentration of 10.[6] 1ml each of the bacterial suspension was obtained with sterile syringe and needle and spread plated with Mueller-Hinton agar. The plates were allowed to stand for 1.5h for the test bacterial isolates to be fully embedded and properly established in the seeded medium. With a sterile cork borer, well of equal depth (Δ = 5mm diameter) were dug with a previously sterilized No 4 cork borer. Each well was aseptically filled up with the respective extracts avoiding splashes and overfilling. The plates were incubated at 37°C for 24 –48h. The sensitivity of the test organisms to each of the extracts were indicated by clear halo around the well. The halos diameter as an index of the degree of sensitivity, were measured with a transparent plastic ruler. Sterile 5% aqueous DMSO was used as negative control while methicillin and streptomycin (10mg/ml) were used as the positive control. The minimum inhibition concentrations (MIC) of the leaf extracts was only determined because they showed higher inhibitory activities of the studied plant. 1ml of the extracts concentrations at 1.25-20mg/ml were mixed with 8ml of Mueller-Hinton broth, 1ml of 24h culture of the test bacterial organisms (1.0x 10^6cells/ml) was inoculated into each test tube of the different concentrations and mixed thoroughly. The test tubes were then incubated at 37°C for 24h. The tube containing the lowest dilution of the extract with no detectable bacterial growth by the naked eye was considered as the point of minimum inhibitory concentration (MIC). One milliliter each of the MIC positive tubes were pour plated with freshly prepared nutrient agar. The plates were incubated for possible growth at 37°C for 96h. Plates without growth were considered as bactericidal concentrations and those with growth as bacteriostatic concentrations of the extracts. All experiments were carried out in triplicates.

Preliminary Photochemical Analysis: The phytochemical was determined using chemical methods and by adopting standard protocols to identify the constituents as described.[21]

RESULTS

A total of 100 patients with different types of wounds samples were collected during the study period. Seven types of bacterial species were isolated and identified by selective culture medium and standard biochemical tests. Each wound samples showed one or more bacterial isolate, totally 78.9% of samples exhibited 24 isolates (Table 1). Among them, *Staphylococcus aureus* (37.2%) was the predominant isolate, second most was *Pseudomonas aeruginosa* (18.6%) followed by *Streptococcus pyogenes* (13.9%) and the lowest percentage was recorded by *Proteus vulgaris* (2.3%). Bacterial isolates were identified using biochemical tests and cultural characteristics.

All bacteria have developed resistance to antibiotics that were once commonly used to treat them. For example, *Staphylococcus aureus* (S7 & S8) *('golden staph')* are now almost always resistant to Methicillin, Carbacillin, Clintamycin, Tetracyclin, Gentamycin. In the past, these infections were usually controlled by Tetracyclin (Table 2). The most serious concern with antibiotic resistance is that all bacteria have become resistant to almost all of the easily available antibiotics. These bacteria are able to cause serious disease and this is a major public health problem. Important examples are methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE) and Erythromycin resistant *E.coli* (ERE).

Table 1: Isolation of bacteria from different types of wound samples

Name of the samples	Name of the isolates							Total	%
	SN3	***SN7***	***SN6***	***SN4***	***SN1***	***SN5***	***SN8***		
Burn	4	-	3	4	1	-	-	12	27.9
Accident wound	3	-	1	1	1	-	1	7	16.3
Abscesses	1	1	-	-	-	-	1	3	7
Skin infection	7	5	4	-	2	1	-	19	44.2
Post operative sepsis	1	-	-	-	1	-	-	2	4.7
Total	16	6	8	5	5	1	2		
(%)	37.2	14	18.6	11.6	11.6	2.3	4.7		

higher antibiotic resistance in biofilm producing bacteria than non-biofilm producers (Table 2).

The next part of the study was prevalence of biofilm formation (Table 3). Among 12 isolates, detected 7 as strong, 1 as moderate and 2 as week biofilm producers. The majority of the organisms associated with biofilm production were *S. aureus* (S7 & S8) followed by *E. coli* (E3), *K. pneumoniae* (Kp2),, *E. faecalis* (En2) and *P. aeruginosa* (Pa2 & Pa7). Very different results were observed by this method, with which only seven isolates showed black colonies with crystalline appearance.

Table 2: Antibiotic susceptibility patterns

Sample Id	M	C	CL	T	G	% of Resistance
S7	R	R	R	R	R	100
S8	R	R	R	R	R	100
St4	R	R	R	R	R	100
St2	R	S	R	R	S	60
Pa2	R	R	R	R	R	100
Pa7	R	R	R	R	R	100
Kp2	R	R	R	S	R	80
Kp4	R	R	R	R	S	80
E3	S	R	R	R	R	80
E4	R	R	R	R	R	100
P1	S	R	S	R	S	40
En2	R	R	R	R	R	100

S-*Staphylococcus sp*, St-*Streptococcus sp* Pa-*Pseudomonas sp*. Kp-*Klebsiella sp*. E-*E.coli*, P-*Proteus sp*. En-*Enterococcus sp*.

M-Methicillin, C- Carbacillin, CL- Clintamycin, T- Tetracyclin, G- Gentamycin.

The leaf extracts of *D. stramonium* were studied for Antibacterial activity and results were observed by presence or absence of zone of inhibition (Table 4). All six extracts aqueous, methanol, acetone, petroleum ether, chloroform and ethanol of *D. stramonium* showed high antibacterial activity. As zone of inhibition were measured in triplicates so mean values±Standard Deviation values were calculated.

Table 3: Biofilm formation of isolates

Isolates name	Biofilm result		
	Strong	Moderate	Week
S7	Positive	Negative	Negative
S8	Positive	Negative	Negative
St2	Negative	Negative	Negative
St4	Negative	Positive	Negative
Pa2	Positive	Negative	Negative
Pa7	Positive	Negative	Negative
Kp2	Positive	Negative	Negative

Kp4	Negative	Negative	Negative
E3	Positive	Negative	Negative
E4	Negative	Negative	Positive
P1	Negative	Negative	Positive
En2	Positive	Negative	Negative

Acetone extract of *D. stramonium* exhibited effective activity against *Staphylococcus aureus* (S7) about 29±0.7 and 28±1.6mm against *P. aeruginosa* (Pa2), 25±0.7mm against *E.coli* (E4) and was 24±0.3mm against *K. Pneumonia* (Kp4).

The ethanol extract showed good activity against *P. aeruginosa* (Pa2) about 25±0.7, 23±0.4mm against *Staphylococcus aureus* (S7) and *Streptococcus pyogene* (St2) 21±0.1mm against *K. Pneumonia* (Kp4) and was 17±0.7mm against *E.coli* (E3).

Among the tested plant extracts acetone extract showed highest activity of 29mm (20mg/ml) zone of inhibition against S7 of *Staphylococcus aureus* followed by ethanol (23mm) and chloroform (16mm). On the other hand, S7 strain were mostly sensitive to various solvents of *Datura stramonium* followed by S8, Pa2, Pa7, E4,Kp4,E3 & En2 STRAIN (Table 4).

The MIC of the *D. stramonium* extracts was determined by agar dilution method. In case of Acetone extract the MIC was 1.56mg/ml against *S. aureus* and 3.12mg/ml against *P. aeruginosa* and 4.25mg/ml against *E.coli*. Ethanol extract exhibited 2.40mg/ml minimum inhibitory concentration against *S. aureus* and 3.12mg/ml against *E.coli* and *P. aeruginosa* and of 12.5 mg/ml against *K. Pneumonia.*

Table 4: Antibacterial Activity of *Datura stramonium* leaves extract

Isolates name	Types of solvents – Inhibition zone in mm									
	AE		CE		PE		EE		ME	
	20	At	20	At	20	At	20	At	20	At
S7	21	8	13	9	15	9	23	9	20	9
S8	22	8	14	8	16	9	18	9	16	9
St2	29	21	16	21	-	21	23	21	-	22
St4	28	21	19	20	-	20	18	21	-	22
Pa2	28	9	18	9	15	9	25	9	18	10
Pa7	25	9	18	9	15	10	22	10	18	10
Kp2	22	17	-	16	13	16	19	16	18	16
Kp4	24	15	-	15	15	15	21	16	19	16
E3	24	10	16	10	-	9	17	9	22	9
E4	25	10	18	10	13	9	15	8	21	9
P1	22	18	17	18	-	18	-	18	15	18
En2	22	16	18	17	-	16	-	17	14	17

AE-Acetone; CE-Chloroform; PE-Petroleum ether; EE-Ethanol; ME-Methanol (mg); At-Antibiotics

Table 5: Preliminary Phytochemical screening of various extracts

Test	Reagents	Results
Saponin	Water shake	Present
Tannin	Lead acetate solution	Absent
Sterol	Acetic anhydride + Sulphuric acid	Present
Terpene	Tin + Thinoyl chloride	Absent
Flavonoid	Mg bits+ HCl	Present
Coumarins	10 % NaoH	Present
Quinone	Conc. H_2SO_4	Present
Lignin	Alc. phloroglucinol + dil.HCl	Absent
Alkaloid	Dragendroff's reagent	Present
Protein	Xanthoproteic test	Negative
Reducing Sugar	Benedict's test	Positive

The phytochemical compounds of *Datura stramonium* extract were analyzed in the present study and the results were showed in Table-5. The phytochemical analysis of *Datura stramonium* showed the presence of alkaloids, steroids, flavonoid, quinine, Coumarins compounds and saponins.

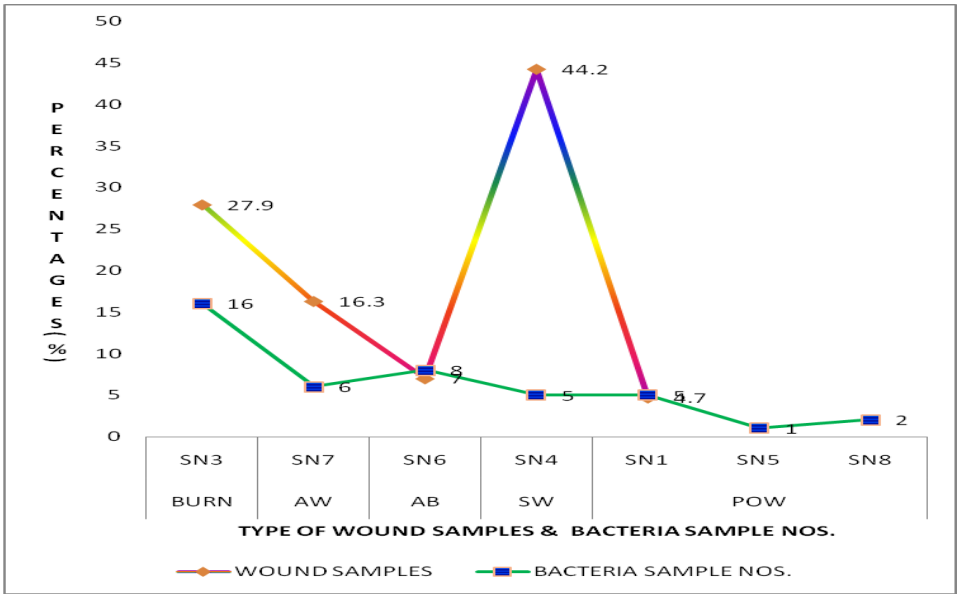

Figure 1:- Isolation of bacteria from different types of wound samples

DISCUSSION

The broad use of traditional medicine by particularly rural Asian communities is attributed to its accessibility and affordability and therefore the use of herbal medicine is becoming progressively more popular worldwide.[22] The prevalence of bacteria in 150 burn wound swabs were shown in the bacterial isolates were found in 100 (66.66%) wound swabs, and only 50 samples (33.33%) were negative in bacterial growth, that contain *P. aeruginosa* was

the commonest isolate (35 isolates; 23.33%) followed by *S. aureus* (15.33%), *Enterobacter* spp (8.66%), *P. vulgaris* (8 %) *Corynebacterium* spp. *E. coli* (4.66 %) And both *Micrococcus* spp and *Klebsiella* spp., (3.33 %).[23] The mean age of the healers (41.93±22.26) was significantly lower (p-value 0.0035) than that of non-healers (63.67±14.19). All non-healing wounds were tested positive for at least one organism by biochemical test, while only 60% of the healing wounds showed the presence of bacterial species. A total of 54 clinically relevant bacterial isolates were identified and the results are Coagulase-positive *Staphylococcus aureus* (56.7%) and *Pseudomonas aeruginosa* (23.3%) were the most prevalent wound bacteria. The prevalence of *Staphylococcus aureus* was significantly higher in non-healing wounds than in healing wounds (p=0.003).[24]

The next part of the study was antibiotic resistance patterns; among the 5 antibacterial agents, Methicillin had highest resistance against *S.aureus* followed by Neomycin against *Streptococcus sp,* Ciproflaxcin of *P.aeruginosa,* Erythromycin of *K.pneumoniae,* Tetracyclin of *E.coli,* Cefataxime of *Proteus sp.* and Nitrofurantain against *Enterococcus faecalis.* Out of 43 species *S.aurues* had highest antibiotic resistance against 6 antibiotics. In case of source wise the highest antibiotic resistance had second most wound infection.[25] Our result was contrary to that report; *S.aurues* had highest antibiotic resistance compared to other isolates. The main factors responsible for virulence of these bacteria are their ability to adhere to host cells, biofilm production. Biofilms are a collection of microorganisms surrounded by the slime they secrete. The ability to form biofilms is associated with the pathogenicity and as such should be considered as an important virulence determinant during wound infection. Biofilms may help maintain the role of bacteria as commensals and pathogen, by evading host immune mechanisms, resisting antibacterial treatment, and withstanding the competitive pressure from other organisms.[26] Consequently, biofilm related infections are difficult to treat. The biofilm production is also associated with high level of antimicrobial resistance of the associated organisms. In this present study all isolates were produce the Biofilm but the same time strongly biofilm producing isolates were high in wound swabs. As predicted by earlier work[27], strongly biofilm isolates had highest antibiotic resistance. In the present study bacterial isolates were studied for their ability to produce biofilm. Biofilm production was more in *S.aureus* than other species. There were no significant differences in biofilm production when grouping the strains according to the patients'.

age, and site of infection. The understanding of microbial biofilm structure and the use of modern technology to bring about modification of the medical devices will lead to decreased microbial infection of medical devices. Antibacterial activity of the plant materials showed different inhibition spectrum against the isolated bacterial pathogens. Administered orally, the antibacterial compounds may be able to control wide range of microorganisms but there is also the posssiblity that they may cause an imbalance in the gut microflora allowing pathogenic coliforms to become established in the wound area with result deleterious effects. Among them leaf acetone extract showed good antibacterial activity compared with other extract. the antibacterial activity of the methanol extracts of the aerial parts of the *D. innoxia* and *D. stramonium*, extracts showed activity against Gram (+) bacteria in a dose dependent manner and little or no antibacterial activity was found against *E. coli*. Present study revealed that *D. stramonium* has maximum antibacterial activity against *Enterobacter* (chloroform extract) and antifunagal activity against *S.cerevicae* (ethanol extract) while it has minimum against *E.coli* (ethanol extract) and *A.niger* (benzene extract). The differences between the results are may be due to use of different solvent for extraction as well as use of different cell culture types.[28] Phytochemical analysis of *Datura stramonium* was due to the presence of phytochemical compounds like alkaloids, tripenoid, steroids, flavonoid, triterpenes, phenolic compounds and tannins.[29] Phytochemical screening of leaf methanol extract of *Datura stramonium* showed positive results for tannins, saponins, flavonoids and alkaloids. From the plant of *Datura metel*, saponin, flavonoids, tannins, phenols and alkakoids glycocides, steroids and terpenoids were identified as phytochemical compounds present. Meanwhile, saponins, flavonoids glycocides, phenols and alkaloids were common to the test plant ethanol and aqueous extracts. Steroid, terpenoids and tannins were absent in the ethanol extract.[30]

CONCLUSION

The preliminary antimicrobial activity of *D. stramonium* studied here can be seen as the potential source of useful drugs. In the present study, the preliminary phytochemical screening of the various chemicals revealed the presence of alkaloids, steroids, flavonoids, saponins, reducing sugar, quinone and coumarins. The plant studied here can be a source of high pharmacological importance and potential source of new drugs. Further studies on such bioactive compounds screening and their antimicrobial activity will unravel the potentiality of these traditional medicines. The promising result obtained has subjected this plant extract to further analyses to screen for its toxicity and side effect for possible perfect therapeutic value.

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