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Full Length Research Paper

Antibacterial and antifungal activity of different extracts of *Datura stramonium* (branches and leaves sample)

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Datura stramonium **branches and leaves sample in three different solvents benzene, chloroform and ethanol was extracted and assessed for antibacterial and antifungal activity. The antibacterial activity was checked against *Enterobacter* (clinical strain/PIMS), *Micrococcus luteus* (clinical strain/PIMS), *Pseudomonas aeruginosa* (clinical strain/PIMS), *E.coli* ATCC 25922, *Staphylococcus aureus* (clinical strain/PIMS) and *Klebsiella pneumonia* ATCC 700603. *Datura stramonium* chloroform extract produced maximum zone of inhibition 16±0.7mm against *Enterobacter* while minimum of 7±0.7mm against *K. pneumonia.* Benzene extract of the plant exhibited maximum of 15±0.7mm of zone of inhibition against *Enterobacter* and *M. luteus* while minimum of 9±0.3mm against *S. aureus* and *K. pneumonia* and ethanol extract of *D. stramonium* gave maximum zone of inhibition against *K. pneumonia* while minimum against *E.coli.* The MBC values revealed that benzene extract (3.12mg/ml) was effective against *P. aeruginosa* while same concentration of chloroform extract was very active against *S. aureus, P. aeruginosa* and *M. luteus.* All the extracts of *D. stramonium* have shown significant antifungal activity against *Saccharomyces cerevisiae, Aspergillus fumigatus and Aspergillus niger* with maximum antifungal activity against *S. cerevisiae* and zone of inhibition was about 16±0.2mm by ethanol extract, 15±0.3mm by chloroform and 14±1.6mm by benzene extract while minimum antifungal activity was observed against *A. niger.***

Key words: *Datura stramonium* extract**,** Zone of inhibition, Antibacterial activity, Antifungal activity, Agar well diffusion method, Minimum inhibitory concentration.

INTRODUCTION

Datura stramonium L., also known as thorn apple, Jamestown weed or devil's trumpet, is a bushy annual belonging to the Solanaceae family (Adams, 1972). It is an erect annual herb forming a bush up to 3–5 ft (1–1.5 m) tall. The leaves are soft, irregularly undulate, and toothed. The fragrant flowers are trumpet-shaped, white to creamy or violet, and 2.5 to 3.5 in. long. They rarely open completely. The egg-shaped seed capsule is walnut-sized and either covered with spines or bald. At maturity it splits into four chambers, each with dozens of small black seeds. Most of the chemical constituents of the plant are found in the whole plant body; however seeds have been reported to contain them in more concentrated form (Stace, 1997).

The plant ingredients are widely used in folklore medicine in the Caribbean and other parts of the world (Morton, 1982). A decoction made from the leaves is taken orally for asthma and sinus infections and the leaves and stripped bark are applied externally to swellings, burns and ulcers (Asprey and Thornton, 1953). The primary psychoactive chemicals in all plants in the *Datura* genus are the alkaloids, scopolamine,

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hyoscyamine and atropine. All parts of the plant are psychoactive but the seeds, leaves, and flowers contain the highest level of alkaloids. The effects of all species of *Datura* are similar when ingested by humans. Seed extract has an analgesic effect in both acute and chronic pain (Khalili and Atyabi, 2004).

D. stramonium is a wild growing plant, used frequently as an anti-asthmatic treatment and it is also known for its hallucinogenic and euphoric effects (Muller, 1998, Weitz, 2003 and Ertekin et al., 2005). This plant not only occurs indigenously in Southern Africa, but also on other areas of the world and was also used by Red Indians for many years as euphoric agent and since the 1800's used as a therapeutic agent and in Great Britain (Dessanges, 2001). However, over dosage can result in severe toxicity. Furthermore, this plant as herbal remedy is also frequently given to pregnant mothers with asthmatic complaints. *D. stramonium* contains a variety of alkaloids including atropine, hyoscamine and scopolamine that can all cause anti cholinergic poisoning if taken in large concentrations (Ertekin et al., 2005); however, these anti cholinergic alkaloids that contribute to the anti-asthmatic properties classified it as a plant with anti cholinergic properties (Friedman, 2004). Atropine is found to have more exciting properties, while scopolamine has more relaxing and hallucinogenic properties (Weitz, 2003). These compounds inhibit or block the physiological action of acetylcholine at a receptor site, and specifically at the muscarinic receptor.

This plant with its two active ingredients affect the unborn baby, as it is known that non-selective anti cholinergic agents such as atropine block both the pre-junctional (M_2), post-junctional (M_3) receptors (Rodrigo and Rodrigo, 2002) as well as the M_1 receptors (Barnes, 2000). Due to the blockade of the M_2 receptors in particular, non-selective antagonists may increase the amount of acetylcholine that is released by cholinergic nerve stimulation (Rodrigo and Rodrigo, 2002).

The plant and fruit are spasmolytic, anti cancerous and anti helmintic. Leaves and seeds are inhaled in whooping cough, asthma and other respiratory diseases. Root, leaf and seed are febrifuge, anti diarrheal, anti catarrhal and are used in insanity, cerebral complications and skin diseases. Leaf is antitumor, anti rheumatic and vermicide. Flower is anti asthmatic, anesthetic and is employed in swellings and eruptions on face. Fruit juice is used in earache and seed decoction in ophthalmia. For the rheumatic swellings of joints, lumbago, sciatica and neuralgia, warm leaf smeared with oil is used as a bandage or sometimes the leaf is made into a poultice and applied (Kaul and Singh, 1995).

It is narcotic, anti spasmodic, anodyne, and ache reliever. It helps in relieving the spasm of the bronchitis in asthma. It is used in treatment of Parkinsonism and hemorrhoids. Young fruits are sedative and intoxicating. Leaves applied after roasting are useful in relieving pain.

Previously various authors have documented antimicrobial activities of medicinal flora of Pakistan (Rizwan et al., 2012, Qayum et al., 2012, Zia-Ul-haq et al., 2011, Nisar et al., 2010a,b; 2011). However many of plants are still unexplored so the objective of present study was to evaluate the antibacterial and antifungal activity of the different extracts of *D. stramonium* branches and leaves sample against selected bacterial and fungal species.

MATERIALS AND METHODS

Sample Collection

The branches and leaves of *D. stramonium* have been collected from various fields of different areas of D.I.Khan. The samples collection was carried out at end of April, were identified from herbarium of faculty of Pharmacy, Gomal University, Dera Ismail Khan. Sample was shade dried and then grinded into powder form.

Extraction

The solvents used for extraction of *D. stramonium* were Benzene 99.5%, Chloroform 99.0-99.4%, Ethanol 99.8%. The 100 grams of the samples were soaked in 300ml of each solvent for 7 days then the soaked samples were filtered out using Whatman No.1 filter paper, then the filterate was evaporated in Rotary Evaporater (Eyela Rotary Vacuum Evaporator Tokyo Rikakikai Co, Ltd) at 40ºC. The vials were washed with detergent and then with acetone and then autoclaved then these vials were used for storing the crude extract. The crude extract was stored into refrigerator for further use.

Antimicrobial Activity Of The Crude Extract

Antibacterial activity was checked by agar well diffusion method (Kavanagh *et al.,* 1963) against six bacterial strains as shown in Table 1.

The turbidity of pure fresh cultures adjusted with 0.5 McFarland turbidity standards and then by sterile cotton swab the lawn of these bacterial cultures were made on nutrient agar plates. Sterile borer were used for making the wells into nutrient agar. Stock solution was prepared by mixing 25mg of the crude extracts of benzene, chloroform and ethanol dissolved in 1ml of DMSO and was added in the respective wells.

Stock solution of each extract individually was used in order to fill these wells. Zone of inhibition was examined after 24 hours and recorded (Espinel- inaroff et.al, 1995, Okeke, 2001). Three reading were taken and calculated the mean result.

Determination of Minimum Inhibitory Concentration (M.I.C)

Agar Dilution Methods

In every test tube 18ml of nutrient agar were taken and autoclaved in the agar dilution method (Mukherjee, 2002). Then added 2ml of crude extract of different concentration 100-

Table 1. Bacterial species used in this experiment

Name	Gram + / -	Shape
Enterobacter	Gram Negative	Rod Shaped
Klebsiella pneumoniae	Gram Negative	Rod Shaped
Escherichia coli	Gram Negative	Rod Shaped
Staphylococcus aureus	Gram Positive	Cocci (Round), Appear as Cluster
Pseudomonas aeruginosa	Gram Negative	Rod Shaped
Micrococcus luteus	Gram Positive	Cocci (Round or Spherical)

0.78mg/ml (given below) into each of separate test tube and then pour it into pre-labeled petri dishes. Another extra blank petri-dish has only nutrient agar was prepared in the same way to compare the growth of respective organisms. The test bacterial strain suspension turbidity was adjusted with 0.5 Mc farland turbidity standard and suspensions of test bacterial strains were transferred into every plate having 100-0.78mg/ml concentration of crude extract. At 37^0C for 24 hours the plates were incubated in incubator observed the plates after 24 hours and smallest concentration which inhibited the growth of respective organism was taken as MIC.

The stock solution of 200mg of extract per ml of DMSO was prepared and then 1ml of DMSO was taken into 8 vials separately. Then taken 1ml from stock solution 200mg/ml and mix it into a vial and then serially diluted it into 8 vials. The serial dilution gave concentration of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.12mg/ml, 1.56mg/ml, and 0.78mg/ml.

Minimum Bactericidal Concentration (MBC)

In order to measure the MBC, it was done by streaking the broth used in MIC on agar plates. There was no growth observation in MBC and it is first dilution MBC sometimes equal or higher then MIC.

Determination of Antifungal Activity

Anti-fungal Activity by Agar Well Method

First of all the stock solution of 30mg/ml of every extracts was prepared. Then the potato dextrose agar (PDA) 20ml were autoclaved and then poured into every petri plate. Then the wells were formed by the help of borer and with stock solution, these wells were filled. At last the petri plates were incubated at 28^0C for 3 days, and zone of inhibition was measured in mm.

Minimum Inhibitory concentration MIC

Agar dilution method was used to determine the MIC of fungal strains. In this method 18 ml of potato dextrose agar were taken in every test tube and then autoclaved in agar dilution method (Mukherjee, 2002). After that 2ml of crude extracts of different concentration 100-0.78mg/ml were added into separate test tubes and then pour it into pre-labeled Petri plates. An extra vacant petri-dish which have only PDA were prepared in same manner to compare the growth of respective fungus. Loopful suspensions of test fungal strains were transferred into every plate having 100-0.78mg/ml concentration of crude extract. Then petri plates were incubated at 28^0C for 3days. After 3 Days, these petri plates were observed and the smallest concentration which inhibited the growth of respective organism was considered as MIC.

RESULTS

Antimicrobial Activity

Antibacterial bioassay

The three different extracts of *D. stramonium* branches and leaves were studied for antibacterial and antifungal activity and results were observed by presence or absence of zone of inhibition. All three extracts benzene, chloroform and ethanol of *D. stramonium* showed high antibacterial and antifungal activity. As zone of inhibition were measured in triplicates so mean values±Standard Deviation values were calculated.

Benzene extract of *D. stramonium* exhibited effective activity against *Enterobacter* and *M. luteus* about 15±0.7 and 12±1.6mm against *P. aeruginosa*, 10±0.7mm against *E.coli* and was 9±0.3mm against *S. aureus* and *K. pneumoniae*.

The Chloroform extract gave 16±0.7mm zone of inhibition against *Enterobacter*, against *S. aureus* and *P. aeruginosa* 14±0.2mm, and 12±1.0mm against *E.coli*, 11±0.7mm against *Micrococcus luteus* and 7±0.7mm against *K. pneumonia.*

The ethanol extract showed good activity against *K. pneumoniae* about 14±1.6mm,12±0.7mm against *Staphylococcus aureus*, 10±0.4mm against *M. luteus*, 8±0.9mm against *E.* and *P. aeruginosa* while 6±0.2mm against *E.coli* (Figure 1).

Minimum Inhibitory concentration MIC

The MIC of the *D. stramonium* extracts was determined by agar d method. In case of benzene extract the MIC was 1.56mg/ml against *M. luteus* and 3.12mg/ml against *P. aeruginosa* and 6.25mg/ml against *Enterobacter, K. pneumoniae, E.coli, S. aureus* (Table 2). Chloroform extract exhibited 1.56mg/ml minimum inhibitory concentration against *S. aureus* and *P. aeruginosa* and 3.12mg/ml against *Enterobacter*, *E.coli* and *M. luteus* and of 12.5 mg/ml against *K. pneumonia* (Table 3).

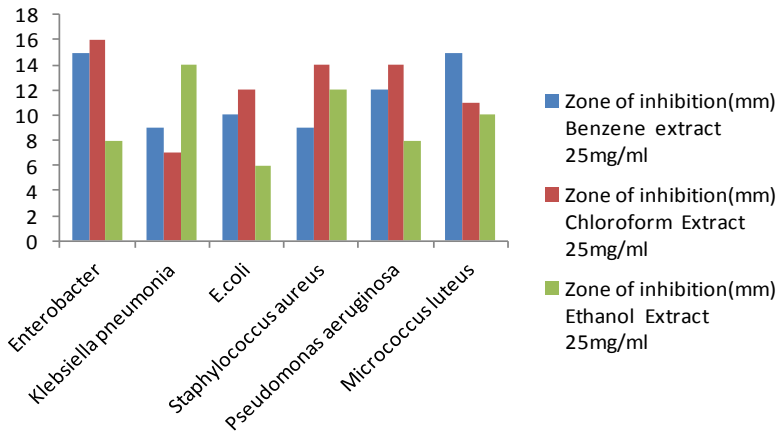

Figure 1. Antibacterial activity of different extract of *Datura stramonium* against selected bacterial species**.**

Incubation temperature: 37^{0}C
Incubation time: 24 hours

Table 2. Minimum Inhibitory Concentration of benzene extract of *Datura Stramonium* against selected bacteria.

Plant Extract	**Minimum Inhibitory Concentration(mg/ml)**							
Benzene Extract	100	50	25	12.5	6.25	3.12	1.56	0.78
Enterobacter	X	X	X	X	X	√	√	√
Klebsiella Pneumoniae	X	X	X	X	X	√	√	√
E.coli	X	X	X	X	X	√	√	√
Staphylococcus aureus	X	X	X	X	X	√	√	√
Pseudomonas aeruginosa	X	X	X	X	X	X	√	√
Micrococcus luteus	X	X	X	X	X	X	X	√

X= No Growth √ =Showing Growth.
Incubation temperature: 37^{0}C
Incubation time: 24 hours

Ethanol extract against *Klebsiella pneumoniae* showed minimum inhibitory concentration of about 1.56mg/ml and 3.12mg/ml against *Staphylococcus aureus*. The concentration 6.25mg/ml *P. aeruginosa* and *M. luteus* while 12.5 mg/ml against *Enterobacter* and *E.coli* (Table 4).

Comparative minimum inhibitory concentration of benzene, chloroform and ethanol extract determined that concentration of 6.25mg/ml of benzene extract is very effective against all bacterial strains (Figure 2).

Minimum Bactericidal Concentration (MBC)

D. stramonium showed very good activity at minimum bactericidal concentration (MBC). The minimum bactericidal concentration of benzene extract against *P. aeruginosa* was 3.12mg/ml and 6.25mg/ml against *K. pneumoniae*, *E.coli* and *M. luteus* and was12.5mg/ml against *Enterobacter* and *S. aureus.*

Chloroform extract exhibited minimum bactericidal concentration of 3.12mg/ml against *S. aureus*, *P. aeruginosa*, and *M. luteus*, 6.5mg/ml against *Enterobacter* and was measured as 12.5mg/ml against *K. pneumoniae* and *E.coli*.

Ethanol extracts showed very good MBC of 3.12mg/ml against *E.coli* and 6.12mg/ml against *K. pneumoniae*, *S. aureus*, *P. aeruginosa* and *M. luteus* and was estimated as 12.5mg/ml against *Enterobacter* (Table 5).

Antifungal Activity

The benzene extract 30mg/ml of *D. stramonium* sample

Table 3. Minimum Inhibitory Concentration of chloroform extract of *Datura Stramonium* against selected bacteria.

Plant Extract	**Minimum Inhibitory Concentration(mg/ml)**							
Chloroform Extract	100	50	25	12.5	6.25	3.12	1.56	0.78
Enterobacter	X	X	X	X	X	X	√	√
Klebsiella Pneumoniae	X	X	X	X	√	√	√	√
E.coli	X	X	X	X	X	X	√	√
Staphylococcus aureus	X	X	X	X	X	X	X	√
Pseudomonas aeruginosa	X	X	X	X	X	X	X	√
Micrococcus luteus	X	X	X	X	X	X	√	√

X= No Growth √ =Showing Growth.
Incubation temperature: 37^{0}C
Incubation time: 24 hours

Table 4. Minimum Inhibitory Concentration of ethanol extract of *Datura Stramonium* against selected bacteria.

Plant Extract	**Minimum Inhibitory Concentration(mg/ml)**							
Ethanol Extract	100	50	25	12.5	6.25	3.12	1.56	0.78
Enterobacter	X	X	X	X	√	√	√	√
Klebsiella pneumoniae	X	X	X	X	X	X	X	√
E.coli	X	X	X	X	√	√	√	√
Staphylococcus aureus	X	X	X	X	X	X	√	√
Pseudomonas aeruginosa	X	X	X	X	X	√	√	√
Micrococcus luteus	X	X	X	X	√	√	√	

X= No Growth √ =Showing Growth.
Incubation temperature: 37^{0}C
Incubation time: 24 hours

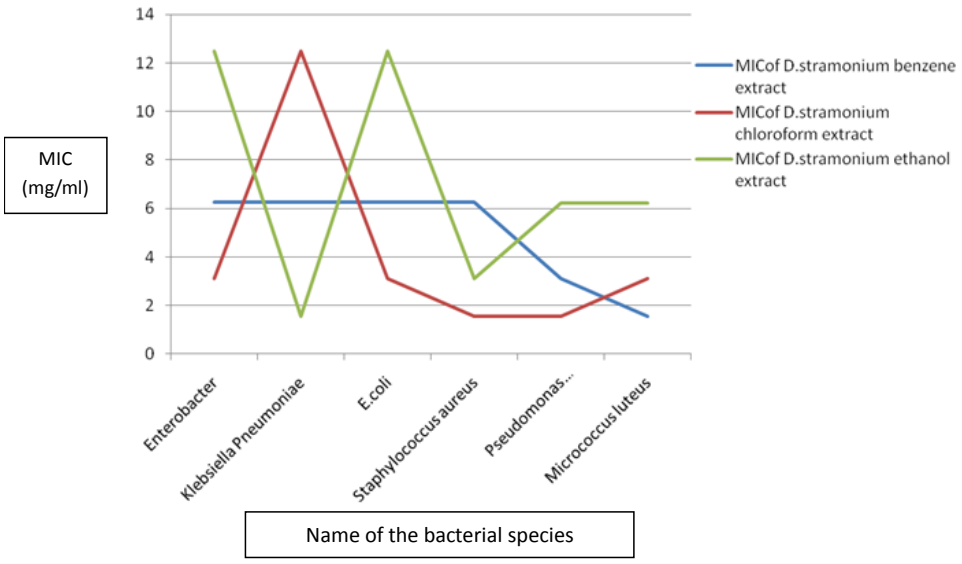

Figure 2. MIC (mg/ml) of different extracts of *Datura stramonium* against tested bacterial strains.

The Minimum concentration which stops growth is MIC.
Incubation temperature: 37^{0}C
Incubation time: 24 hours

Table 5. Minimum Bactericidal Concentration of different extracts of *Datura Stramonium*

Bacteria	Benzene	Chloroform	Ethanol
Enterobacter	12.5mg/ml	6.25 mg/ml	12.5 mg/ml
Klebsiella pneumoniae	6.25 mg/ml	12.5 mg/ml	6.25 mg/ml
E.coli	6.25 mg/ml	12.5 mg/ml	12.5 mg/ml
Staphylococcus aureus	12.5 mg/ml	3.12 mg/ml	6.25 mg/ml
Pseudomonas aeruginosa	3.12 mg/ml	3.12 mg/ml	6.25 mg/ml
Micrococcus luteus	6.25 mg/ml	3.12 mg/ml	6.25 mg/ml

Incubation temperature: 37^0C
Incubation time: 24 hours

Table 6. Antifungal activity of different extracts of Datura stramonium against selected fungi.

Fungal Strains	Concentration	Zone Of Inhibition Mean± Standard Deviation (mm)		
		Benzene	Chloroform	Ethanol
Aspergillus niger	30mg/ml	8±0.4	9±0.2	10±0.3
Aspergillus fumigatus	30mg/ml	12±0.9	11±0.4	13±0.7
Saccharomyces cerevisiae	30mg/ml	14±1.6	15±0.3	16±0.2

showed high activity against *S. cerevisiae* about 14±1.6mm, 12±0.9mm against *A. fumigatus*, and 8±0.4 mm against *A. niger*.
The chloroform extract exhibited great activity against *S. cerevisiae* about 15±0.3 mm, 11±0.4 mm against *A. fumigatus* and 9±0.2 mm against *Aspergillus niger* zone of inhibition.

The ethanol extract was found as highly effective against the three fungal strains and zone of inhibition measured against *S. cerevisiae* was about 16±0.2mm, 13±0.7mm against *A. fumigatus* and was10±0.3mm against *A. niger* (Table 6).

Minimum Inhibitory concentration of fungal strains

By agar Dilution method the MIC of the fungal strains were determined. The MIC of chloroform and ethanol extracts against *S. cerevisiae* was 3.12mg/ml while of benzene extract was found as 6.25mg/ml. The MIC of both benzene and ethanol extracts against *A. fumigatus* was 6.25mg/ml while of chloroform extract against this strain was 12.5mg/ml. The MIC of chloroform, benzene and ethanol extracts against *A. niger* was found as12.5mg/ml.

Comparative result of MIC of *D. stramonium* extracts against selected fungi described that minimum concentration of 12.5mg/ml of all the three extracts is quite effective in the inhibition of growth of *Aspergillus niger* (Figure 3).

DISCUSSIONS

The broad use of traditional medicine by particularly rural African communities is attributed to its accessibility and affordability and therefore the use of herbal medicine is becoming progressively more popular worldwide (Steenkamp, 2003).

Tannins and alkaloids were present and that the ethyl acetate and methanolic leaf extracts of the plant were active against *E. coli, P. vulgaris*, *K*. *pneumonia* and *A. niger* (Adebayo et al, 1989). All parts of the plants were strongly intoxicant and narcotic. Saponins are a special class of glycosides which have soapy characteristics (Fluck, 1973). It has also been shown that saponins are active antifungal agents. This supports the earlier finding that extracts of the plants used in the present work may be useful in the chemotherapy of mycotic infections. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Sodipo et.al., 1991). Classes of alkaloids are among the major powerful poisons known (Fluck, 1973). Apart from being poisonous, some alkaloids have also been proved to be useful in correcting renal disorders (Konkwara, 1976); it therefore, means that the alkaloids of *Angraecum. mauritianum*, *Bacopa monnifera* and *D. stramonium* may be a poison that can be tried on lower or higher organisms. The secondary metabolites identified in the plant materials used in this study could be responsible for antimicrobial activity exhibited by these plants.

Antimicrobial activity of *D. stramonium* was due to the presence of phytochemicals. Eftekhar et al. (2005) reported 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

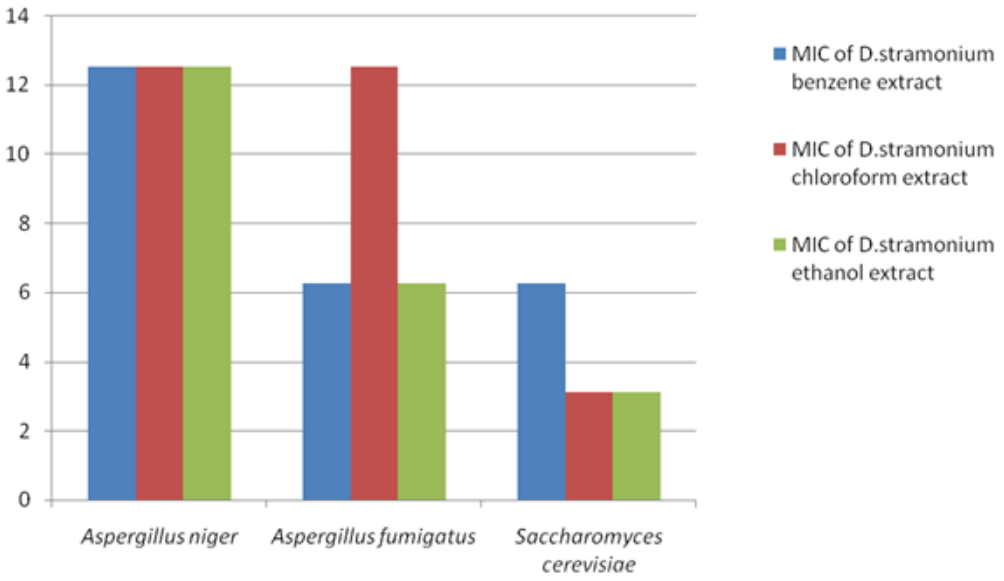

Figure 3. MIC (mg/ml) of different extracts of *Datura stramonium* against selected fungi.

The Minimum concentration which stops growth is MIC.
Incubation temperature: 28^{0}C
Incubation time: 72 hours

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.

Conclusion

The preliminary antimicrobial activity of *D. stramonium* studied here can be seen as the potential source of useful drugs. Different extracts showed varying degrees of antibacterial and antifungal activities against microbes tested here. 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.

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