ANTIMICROBIAL ACTIVITY OF *DATURA STRAMONIUM* L. AND *TYLOPHORA INDICA* (Burm.f.) Merr.

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Summary

The alcoholic extract of *Datura stramonium* L. (Solanaceae) and *Tylophora indica* (Burm.f.) Merr. (Asclepiadaceae) were evaluated for antimicrobial activity. The dried leaves of these plants were extracted with 95% alcohol. The preliminary phytochemical investigation was done for both the extracts to identify various phytochemical constituents present in the extracts and also subjected to antimicrobial activity for the assessment of inhibitory effects of the alcoholic extracts of these plants against nine medically important pathogenic microbes by *in vitro* agar well diffusion method. The results of the preliminary phytochemical studies revealed the presence of alkaloids, phenols, flavonoids, tannins, steroids, saponins in both the plants. In addition to this, these extracts exhibited significant zone of inhibition and good antimicrobial activity against the majority of the selected strains of microorganisms, such as *Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger* and *Fusarium* species. These present results leading to the conclusion that these plants would serve as sources novel antimicrobial agents.

Key Words: *Datura stramonium, Tylophora indica*, Agar Well Diffusion

Introduction

Control of microorganisms is critical for the prevention and treatment of disease. In recent years many microbial diseases like chicken guniya, dengue fever, AIDS etc. have become challenging to the modern medical world. A vast majority of synthetic antibiotics controls the growth and development of microorganisms effectively, but they are highly toxic at their optimum dosage level. Among many proposed strategies, a good understanding of plants offers the potential of developing potent broad spectrum antibiotics. Hence, the present research was undertaken to evaluate the antimicrobial activity of *Datura stramonium* and *Tylophora indica.*

Datura stramonium L (Solanaceae) commonly called as thorn apple. It is large and coarse herb, through an annual, branching somewhat freely, giving bushy look to the plant. It attains a height of about 3 feet with simple leaves and white to pale coloured flowers. It contains some of the active principles like hyoscyamine, scopolamine and atropine. It is used to cure ulcers, scabies, leprosy, piles, anemia, fever and inflammatory swellings, elephantiasis etc. *Tylophora indica* (Burm.f.) Merr. (Asclepiadaceae) commonly called as Indian Ipecac, it is a twining perennial herb with spreading branches, ovate leaves and greenish yellow coloured flowers. It is used to treat bronchial asthama, bronchitis asthama, rheumatism and dermatitis etc. (1-3)

Materials and Methods

Collection of Plant materials: The leaves of *D. stramonium* and *T. indica* were collected from in and around Gulbarga city, Karnataka, India. All these plants were authenticated and the voucher specimens were deposited in Herbarium of Department of Botany, Gulbarga University, Gulbarga, India. Later these plant leaves were subjected for surface sterilization using 50% alcohol, and then shade dried for further analysis.

Preparation of the Extracts: The 100g leaves of *D. stramonium* and *T. indica* were extracted with 95% alcohol at 50 - 60°C in a soxhlet apparatus. The different extracts were collected in a separate container and concentrated to dryness in a flash evaporator (Buchi type) under reduced pressure and controlled temperature (40 - 50°C) and note down the yield of crude extracts.

Microorganisms Used: Clinical laboratory bacterial isolates of *Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger, Aspergillus fumigatus* and *Fusarium* species were collected from the stock cultures of Microbiology Laboratory, Basaveshwara Hospital,

Gulbarga, Karnataka, India. The bacterial and fungal cultures were maintained on nutrient agar and SDA agar medium respectively, and were stored at 4˚C, for determining antimicrobial activity of some medicinal plants.

Culture Media: The nutrient agar, nutrient broth for antibacterial activity and Sabouraud's dextrose agar for antifungal activity were purchased from HiMedia, Laboratories Limited, Mumbai. Nystatin an antifungal agent, purchased from, Tocelo chemicals, Netherlands, Streptomycin sulphate (powdered form) another antibiotic from Nanjing Asian chemicals Co., Ltd. The solvents and other chemicals used were analytical grade.

The Preliminary Phytochemical Studies: The alcoholic extracts of *D. stramonium* and *T. indica* were tested by applying general chemical tests for alkaloids, glycosides, reducing sugars, tannins, steroids, terpenoids, phenols, flavonoids, proteins, saponins, amino acids, etc (4 -8).

Characterization of Microorganisms: The identity of all the above mentioned microorganisms was confirmed by subjecting these organisms with certain confirmatory tests, such as Gram staining, bacterial motility, colony characteristics, biochemical tests, such as coagulase test, sugar fermentation test, indole production, urease production, citrate utilization teat, catalase, oxidase tests (9) . The results of all the above tests were compared with the standard authentic sources to confirm the identity of these organisms (10-11).

Antimicrobial activity: Here the *in vitro* antibacterial and antifungal activities were assayed by using agar well diffusion method. The pure cultures of different pathogens were grown overnight in sterile nutrient broth and incubated at 37°C for 24 hours, then subjected for optical density of the overnight incubated culture were adjusted to 0.1 at λ_{600} with sterile nutrient broth. The 0.1ml of the culture was seeded on 25 ml of solidified nutrient agar plate and Sabouraud's dextrose agar plates for bacterial and fungal cultures. The wells were bored with 8mm borer in seeded agar, and then the particular concentrations (4mg/0.2ml to 8mg/0.2ml) of the plant extracts and 500µg of streptomycin sulphate and Nystatin were added in each separate well. Soon after the plates were then kept at 10°C for 30min. After it normalized to room temperature plates were incubated at 37°C for 24hr. Later, the zone of inhibition was measured and recorded (12-13).

Statistical Analysis: All the data are expressed as mean ± S.E.M. (standard error of the mean). The significance level was determined using the Student's't' test. A *p*-value of <0.05 was considered statistically significant.

Results

Preliminary Phytochemical studies: The preliminary phytochemical studies revealed the presence of alkaloids, glycosides, reducing sugars, tannins, steroids, terpenoids, phenols, flavonoids, proteins, saponins, amino acids, etc in both the plant extracts.

Characterization of Microorganisms: All the above tested bacterial forms are Gram negative and rod shaped, except *Staphylococcus aureus* is Gram positive and perfectly spherical in shape, where as all these bacterial forms are proved to be motile, except *Klebsiella pneumoniae* and *Staphylococcus aureus* are non-motile forms. Results of certain confirmatory biochemical tests for these bacterial forms are also explained in the Table-1. Where as the microscopic observations of the fungal forms revealed that, the *Aspergillus niger* has septate branching mycelia, the conidia are black coloured and thus appear as black colour mould. Similarly, *A. fumigatus* produces septate branching mycelia, the conidia are green coloured and thus appear as green mould. Where as, *Fusarium* species produces hyaline branched, geniculate septate mycelia. Based on these unique properties, identities of these organisms were confirmed.

Antimicrobial activity: The alcoholic extract of *D. stramonium* (4, 6, and 8mg/ 0.2ml) has shown promising antimicrobial activity and effectively inhibited the growth rate of *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger* and *Fusarium* sps, but *Klebsiella pneumoniae* and *Proteus vulgaris* were showed resistance to different concentrations of the *Datura stramonium* leaf extract. (Table-2) The different concentrations of the alcoholic extract of *T. indica* has shown potent antimicrobial activity and significantly inhibited the growth rate of microorganism viz *Aspergillus niger, Pseudomonas aeruginosa, Proteus vulgaris,* mild to moderate activity was noticed against *Escherichia coli, Aspergillus fumigatus* and *Fusarium* species at lower concentrations (4mg/0.2ml) of the extract. Where as significant antimicrobial effect was noticed against all the above mentioned pathogens. However, *Klebsiella pneumoniae* and *Salmonella typhi* were showed resistance to different concentrations of *T. indica* extract (Table-3).

Table-1 Microscopic and Biochemical profile of Clinical isolates

Microorganisms	Gram Staining	Motility	Structure	Sugar fermentation test	Indole production test	Urease production test	Citrate utilization test	Catalase test	Oxidase test
Klebsiella pneumoniae	Negative	Non-motile	Rod shaped	AG	Negative	Positive	Negative	Negative	Negative
Staphylococcus aureus	Positive	Non-motile	Spherical shaped	A	Negative	Negative	Negative	Positive	Negative
Salmonella typhi	Negative	Motile	Rod shaped	Negative	Negative	Negative	Positive	Positive	Negative
Proteus vulgaris	Negative	Motile	Rod shaped	AG	Positive	Positive	Negative	Positive	Negative
Pseudomonas aeruginosa	Negative	Motile	Rod shaped	Negative	Negative	Positive	Positive	Positive	Positive
Escherichia coli	Negative	Motile	Rod shaped	A	Positive	Positive	Positive	Negative	Negative

Note: AG – Acid and Gas; A –Acid

Table-2 Antimicrobial activity of ethanolic extract of *Datura stramonium* L.

Microorganisms	Zone of inhibition (mm)					
	Control (0.2ml of d.w)	4mg / 0.2ml	6mg / 0.2ml	8mg / 0.2ml	500µg / 0.2ml (Streptomycin sulphate)	500µg / 0.2ml (Nystatin)
Klebsiella pneumoniae	Nil	Nil	Nil	Nil	09.31±1.97	NT
Staphylococcus aureus	Nil	7.0 ±1.00	7.25±0.66	8.0±0.50	11.80±2.39	NT
Salmonella typhii	Nil	6.5 ±0.50	7.00±1.00	7.25±0.26	08.10±0.62	NT
Proteus vulgaris	Nil	Nil	Nil	Nil	09.25±0.67	NT
Pseudomonas aeruginosa	Nil	7.5 ±0.50	7.75±0.30	9.75±0.75	10.00±0.00	NT
Escherichia coli	Nil	7.0 ±1.00	7.75±0.90	8.75±0.86	11.25±2.10	NT
Aspergillus fumigatus	Nil	Nil	Nil	Nil	NT	10.60±2.75
Fusarium sp.	Nil	7.5 ±0.30	8.00±0.20	8.50±0.34	NT	12.18±0.55
Aspergillus niger	Nil	7.0±0.20	8.00±0.43	8.50±0.45	NT	11.25±0.86

Note : dw =distilled water; NT –Not tested; Nil – no activity

Table-3 Antimicrobial activity of ethanolic extract of *Tylophora indica* (Burm.f.) Merr.

Microorganisms	Zone of inhibition (mm)					
	Control (0.2ml of d.w)	4mg / 0.2ml	6mg / 0.2ml	8mg / 0.2ml	500µg / 0.2ml (Streptomycin sulphate)	500µg / 0.2ml (Nystatin)
Klebsiella pneumoniae	Nil	Nil	Nil	Nil	09.31 ± 1.97	NT
Staphylococcus aureus	Nil	7.00 ±1.00	07.75±0.75	08.00 ± 0.50	11.80 ± 2.39	NT
Salmonella typhi	Nil	Nil	Nil	Nil	08.10 ± 0.62	NT
Proteus vulgaris	Nil	7.50 ± 0.50	08.00 ± 0.30	09.00 ± 1.00	09.25 ± 0.67	NT
Pseudomonas aeruginosa	Nil	8.00 ± 1.13	09.50 ± 1.18	09.75 ± 0.90	10.00 ± 0.00	NT
Escherichia coli	Nil	5.75 ± 0.06	06.75 ± 0.06	08.00 ± 0.75	11.25 ± 2.10	NT
Aspergillus fumigatus	Nil	6.50 ± 1.41	07.50 ± 0.60	08.50 ± 0.70	NT	10.60±2.75
Aspergillus niger	Nil	6.50 ± 1.69	07.00 ± 0.20	09.00 ± 0.20	NT	12.18±0.55
Fusarium sp.	Nil	9.00 ± 0.90	10.00 ± 1.00	10.50 ± 0.70	NT	11.25±0.86

Note : dw =distilled water; NT –Not tested; Nil – no activity

Discussion

The different concentrations of the alcoholic extracts of *D. stramonium* and *T. indica* were proved to be effective and concentration dependent antimicrobial activity against both Gram positive and Gram negative bacteria and fungi tested. This is evidenced by several research groups, are also supporting the presence of antibacterial activity of *D. stramonium* against Gram positive bacteria in methanolic extract of a dose dependent manner (14). The crude ethanolic extract and total alkaloids of *D. stramonium* showed good antibacterial activity against four microbial species, *Escherichia coli, Staphylococcus aureus, Staphylococcus saprophyticus, Pseudomonas aeruginosa* the *in vitro* agar well diffusion method was used (15). Obic *et al*, (2002) (16) have shown the antibacterial activity of *Datura stramonium* against most of the Gram positive and Gram negative bacteria. Among *Pseudomonas aeruginosa* and *Proteus vulgaris* were observed to be completely resistant to the extracts of *Datura stramonium.* These studies strongly supports to the present research. So far there are no reports on the clinical antibacterial and antifungal activity on *Tylophora indica.*

Conclusion

The alcoholic extracts of both the plants were found to exhibit greater non-specific, broad spectrum antimicrobial activity. Phytochemical evaluation revealed the presence of alkaloids, phenols, flavonoids, tannins saponins in these plants have been claimed to be responsible for their antimicrobial activity. Hence leading to the conclusion that, these plants would serve as sources of novel antimicrobial agents.

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