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PLEBEIAN ASSESSMENT OF ANTIMICROBIAL AND *IN VITRO* ANTIOXIDANT ZEST OF *DATURA FASTUOSA* L. SEEDS

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ABSTRACT
The antimicrobial zest of hydro-alcoholic and methanolic seed extracts of *Datura fastuosa* L. was evaluated against three clinical bacterial strains (*Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis*) and two clinical fungal strains (*Candida allbicans* and *Aspergillus niger*) by tube dilution method. Both the plant extracts were found to be active against the tested micro-organisms. The methanolic extract of *D. fastuosa* was found to inhibit bacterial strain, *E.coli* effectively with minimum bactericidal concentration (MBC) of 25µg/ml. The hydroalcoholic extract of *D. fastuosa* seeds was found to be more potent in terms of its bactericidal concentration for *B. subtilis* with both minimum inhibitory concentration (MIC) and MBC values as 25 µg/ml. Also, methanolic extract was found to be more efficient in inhibiting *S. aureus* having MIC 12.5 µg/ml. Hydro alcoholic and methanolic seed extracts of *D. fastuosa* were also evaluated for antioxidant potential using 1,1-diphenyl-1-picryl-hydrazyl radical scavenging assay, total antioxidant capacity, total amount of phenolic content, total flavonoid content, total flavonols and total proanthocyanidines content. The IC_{50} value using DPPH model for methanolic extract of *D. fastuosa* was found to be 28.34 µg/ml & for hydroalcoholic extract, 25.78 µg/ml. The observed values for total antioxidant capacity, total amount of phenolic content, total flavonoid content, total flavonols and total proanthocyanidines content for methanolic extract of *D. fastuosa* were found to be 6.83 mg/g, 9.97 mg/g, 6.34 mg/g, 5.37 mg/g and 1.42 mg/g of plant extract respectively and for hydroalcoholic extract, the values for the same parameters were 7.44 mg/g, 6.88 mg/g, 9.35 mg/g, 5.36 mg/g and 0.88 mg/g respectively.
KEYWORDS: Antibacterial, antifungal, antioxidant, *Datura fastuosa* L.

INTRODUCTION

Medicinal plants are used for the ailment of several microbial and non-microbial originated diseases due to their valuable effects in health care. The reliability, availability and lower side effect of medicinal plants in therapeutic use has made them popular and acceptable in medical health care all over the world. Several plants have therapeutic and pharmaceutical effects for antimicrobial, antioxidant, anti-infectious and antitumour activities. [1, 2] Active chemical constituents from medicinal plant has been most widely used as primary health care in many countries.[3]

Datura fastuosa Linn, also known as *D. metel* or *D. alba* Nees (Solanaceae) is a Nigerian medicinal plant mostly used in phytomedicine, particularly the leaves and seeds are used as anesthetic, anodyne, anti-asthmatic, anti-pasmodic, antimicrobial, anti-tussive, bronchodilator, and hallucinogenic.[4]

As per the reported literature, antimicrobial activity of ethyl acetate and methanol extracts of *D. metel* plant was investigated by agar disc and well-diffusion method against HIV associated opportunistic infections. The plant extracts showed better inhibitory activity against the tested organisms.[5] Potential antibacterial activity of various parts of *D. innoxia* were examined by preparing their crude aqueous and organic extracts against gram negative bacteria (*E. coli* and *S. typhi*) and gram positive bacteria (*B. cereus*, *B. subtilis* and *S. aureus*).[6]

In a study, *D. metel* leaf extracts significantly reduced the *in vitro* growth of *Rhizoctonia solani* and *Xanthomonas oryzae* pv. O*ryzae*. The methanolic extract showed the best control of the pathogens recording 10–35% more toxicity than aqueous extract. Foliar application of leaf extracts effectively reduced the incidence of sheath blight and bacterial blight diseases of rice under green house condition.[7]

The antibacterial activity of methanolic and hexane leaf extracts of *D. stramonium, Ricinus communis, Calotropis gigantean, Malva verticillata* and *Malvastrum coromandelium* was evaluated against *E. coli*, *S. aureus* and *B. subtilis* by agar well diffusion method. The maximum antimicrobial activity expressed in terms of zone of inhibition was shown by methanolic and hexane leaf extracts of *D. stramonium* followed by *Ricinus communis, Calotropis gigantean* to and *Malva verticillata* against the tested organisms. This study also revealed that methanolic leaf extract was more effective than hexane extracts in term of their antibacterial potential.[8]

The antibacterial activity of the methanol extracts of the aerial parts of the *D. innoxia* and *D. stramonium* was investigated and the extracts showed activity against gram (+) bacteria in a dose dependent manner. [9]

The antimycotic potential of *D. metel* was also investigated *in vitro* against *Ascochyta rabiei,* the cause of chickpea blight disease. The pathogen was exposed to various n-hexane concentrations (1, 2, 3 and 4% w/v) of shoot and root extracts of *D. metel* using poisoning food technique. All the tested concentrations of both root and shoot extracts inhibited the growth of the target fungal pathogen.[10]

A novel compound 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylethyl pentanoate was isolated from the plant *D. metel* L. The *in vitro* activity of this dihydropyrrole derivative against *Aspergillus* and *Candida* species was evaluated by using standard methods approved by the National Committee for Clinical Laboratory Standards. The compound was found to be active against all the species tested, namely *C. albicans, C. tropicalis, A. fumigatus, A. flavus* and *A. niger*.[11]

A steroidal compound, withametelin, isolated from *D. metel* leaves showed antifungal activity against some plant pathogenic and saprophytic fungi.[12]

Antioxidant potential was found to be higher in *D. metel* leaf than bark. The aqueous extract of *D. metel* leaf and stem bark showed antioxidant effect of 48.80% and 23.82% respectively, while the ethanol extract showed 25.51% and 3.41%.[13]

MATERIALS AND METHODS

Chemical and Reagents Used

Sodium chloride, glucose, peptone, yeast, agar, dimethyl sulphoxide (DMSO), methanol, ascorbic acid, potassium ferricyanide, phosphate buffer, DPPH, ferric chloride, o-phenanthroline, ethanol, FC (Folin Ciocalteu) reagent, gallic acid, sodium phosphate, ammonium molybdate, aluminium trichloride, sodium acetate, quercetin, vanillin. All the chemical and reagents used in the present study were of analytical grade.

Authentication of Plant part

Selected plant seeds i.e. *D. fastuosa* seeds were collected from Khari Baoli market, New Delhi and authenticated at I.A.R.I.(NISCAIR) Pusa Road, New Delhi, by Dr. H. B. Singh, Head, Raw material herbarium and museum division, NISCAIR, New Delhi.

Extraction of *Datura fastuosa* Seeds

The plant seeds were air dried for 3 weeks and then ground to coarse powder using a mechanical grinder. The powder obtained was extracted with methanol and water:ethanol (1:1) separately at room temperature (25±2^{0}C). The crude plant extracts were obtained by using soxhlation. The obtained semi-dried crude extracts were labelled appropriately as ME (methanol extract) and HAE (hydroalcoholic extract) and kept in desiccator for further use.

Microorganism collection

The bacterial and fungal strains selected for present study were obtained from Department of Microbiology, Guru Jambeshwar University, Hissar, India. A total of three bacterial strains namely *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* & two fungi namely *Candida albicans* and *Aspergillus niger* were screened for antimicrobial zest. These bacterial cultures were maintained in nutrient agar slants at 37°C. The microorganisms were reactivated prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37°C.

Media preparation

Double strength nutrient media used for antibacterial studies was prepared using peptone (1g), yeast (0.3g), sodium chloride (0.5g) and dissolving these ingredients in water q.s. to make 50 ml. The media was then sterilized by autoclaving at 15 lb/Psi pressure for 15 min. Double strength Sabouraud's glucose broth used for antifungal studies was prepared by dissolving glucose (8g), peptone (2g) in distilled water q.s. to make 100ml with aid of heating. Then the medium was cooled and filtered, pH was adjusted to 5.4 with 10% lactic acid. The media was sterilized by autoclaving at 15 lb/Psi pressure for 15 min. [14]

Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

Turbidimetric method or tube dilution method was used for determination of minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration. 1 ml of the sterilized media poured in the concentration of 50 µg/ml was used. The extract was serially diluted to give a concentration of 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 µg/ml. In all the test tubes, 0.1 ml of suspension of bacteria in saline was added and incubated at 37^{0}C for 24 h (for plates containing bacterial cultures), 37^{0}C for two days (for plates containing *Candida albicans* culture) and 25^{0}C for seven days (for plates containing *Aspergillus nige*r culture). A tube containing nutrient broth only was seeded with the test organism to serve as control. All the tubes were then incubated at 37^{0}C for 24 h and then examined for growth by observing turbidity. The minimum bactericidal concentration (MBC) of the plant extract on the clinical bacterial isolates was carried by pipetting out 0.1 ml bacterial culture from the mixture obtained in the determination of MIC tubes which did not show any growth and sub cultured on to nutrient media and incubated at 37^{0}C for 24 h. After incubation the concentration at which there was no single colony of bacteria was taken as MBC. MFC was also determined similar to MBC. [15]

In vitro antioxidant studies

Different concentrations of both methanolic and hydroalcoholic extracts of *D. fastuosa* seeds (2, 4, 8, 16, 32, 64, 128, 256, 512 and 1000 µg/ml) in DMSO were prepared and then proceeded for antioxidant zest determination. Following parameters were studied.

1. 1,1-diphenyl-1-picryl-hydrazyl radical scavenging assay (DPPH): 0.0002 % DPPH in methanol was prepared. Added 1 ml DPPH in all drug solutions of different dilutions. These were kept in dark for half an hour and then absorbance was taken using double beam spectrophotometer at 517 nm. The same procedure was followed for the standard drug, ascorbic acid. Percentage inhibition and IC_{50} were calculated using the formulae. [16]

$IC_{50} = a + b\,(50)$

$$b = \frac{\sum x.y}{\sum x^2}$$

$$a = \bar{y} - b\,\bar{x}$$

where a= intercept on the line
b= regression coefficient of x on y
x = concentration in µg/ ml
y = % scavenging

$$\%\text{scavenging} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

2. Total phenolic and polyphenolic content determination: Prepared 300 µl drug extract, added 1 ml Folin Ciocalteu's reagent (FC reagent) and 5 ml sodium carbonate (20%). Final volume was made upto 10 ml with distilled water and absorbance was taken at 735 nm. Same procedure was followed for its standard gallic acid. [17]

3. Total antioxidant capacity: 0.1 ml of 10 mg/ml drug extract was taken and 1ml reagent consisting of 0.6 M H_2SO_4, 28 mM Na_2PO_4, 4 mM ammonium molybdate was added. The tubes were covered with caps and incubated at 95º C for 90 min. Then they were allowed to cool and absorbance was taken at 695 nm. [18]

4. Total flavanoid content : 0.5 ml of each extract solution was taken and added 0.5 ml $AlCl_3$ (2%). After 1 h incubation, the absorbance was measured at 415 nm. Same procedure was followed for standard, gallic acid. [19]

5. Total flavonols : To 2ml of 1000 µg/ml drug extract, 2 ml $AlCl_3$ (2% in ethanol) and 3 ml sodium acetate (50 g/l) was added. After 2.5 h, absorbance was taken at 440 nm. Same procedure was followed for standard, quercetin.[19]

6. Total pro-anthocyanidines content: To 0.5 ml of 0.1 mg/ml extract solution, 3 ml of 4% vanillin methanol was added. 1.5 ml of HCl was added, allowed to stand for 15 min and absorbance was taken at 500 nm. [20]

RESULT AND DISCUSSION

Antimicrobial activity

In the present investigation, results showed that both methanolic and hydroalcoholic extract of *D. fastuosa* inhibited the growth of bacteria *S. aureus, E. coli, B. subtilis*, and the fungus *C. albicans* and *A. niger* by tubidimetric method.

It was found that both methanolic and hydroalcoholic extracts of *D. fastuosa* exhibited antimicrobial activity against tested microbial strains. However, hydroalcoholic extract of *D. fastuosa* seeds was found to be more potent in terms of its bactericidal concentration for *B. subtilis*, when compared with methanolic extract. Also, methanolic extract was found to be more efficient in inhibiting the *S. aureus*, than hydroalcoholic extract (Table 1, 2).

Table 1: MIC and MBC/MFC of methanolic extract of *D. fastuosa* seed against microbial strains (bacterial and fungal)

Microbial strains (Bacteria and fungi)	MIC (in µg/ml)	MBC or MFC (in µg/ml)
Bacillus subtilis	25	50
Staphylococcus aureus	12.5	50
Escherichia coli	12.5	25
Candida albicans	12.5	>50
Aspergillus niger	12.5	>50

Table 2: MIC and MBC/MFC of hydroalcoholic extract of *D. fastuosa* seed against microbial strains (bacteria and fungi)

Microbial strains (Bacteria and fungi)	MIC (in µg/ml)	MBC or MFC (in µg/ml)
Bacillus subtilis	25	25
Staphylococcus aureus	25	50
Escherichia coli	12.5	25
Candida albicans	12.5	>50
Aspergillus niger	12.5	>50

DPPH assay

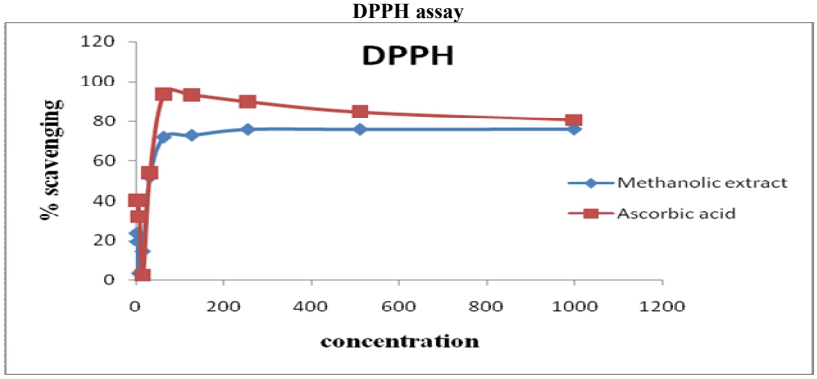

Fig 1. Antioxidant activity of methanolic extract by DPPH method

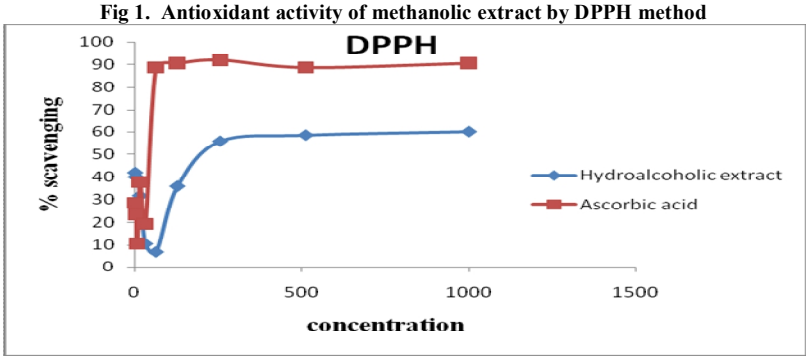

Fig 2. Antioxidant activity of hydroalcoholic extract by DPPH method

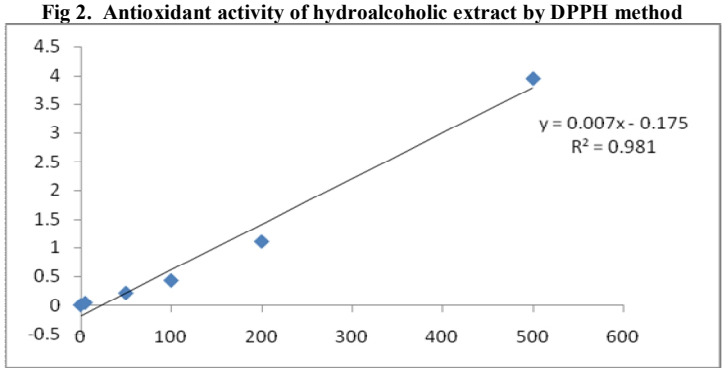

Fig 3. Standard curve of ascorbic acid

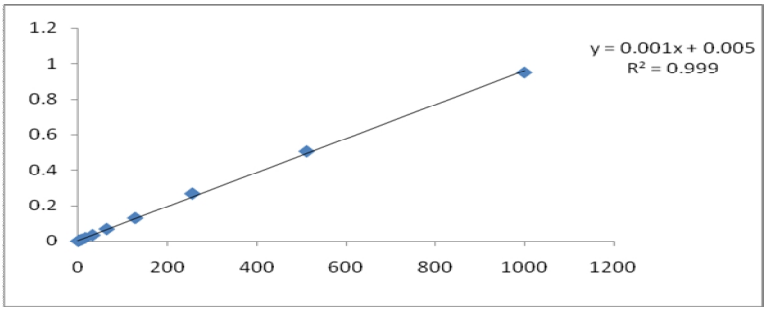

Fig 4. Standard curve of gallic acid

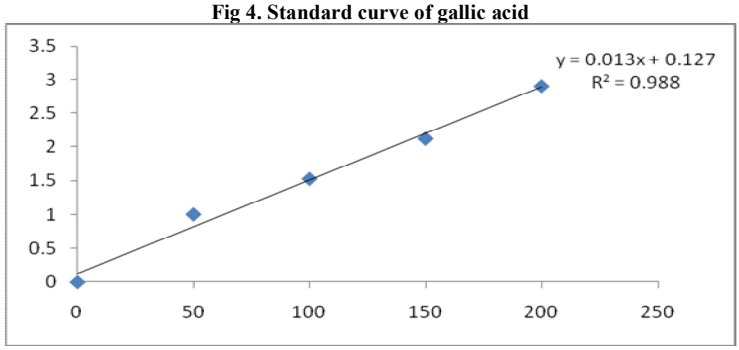

Fig 5. Standard curve of gallic acid

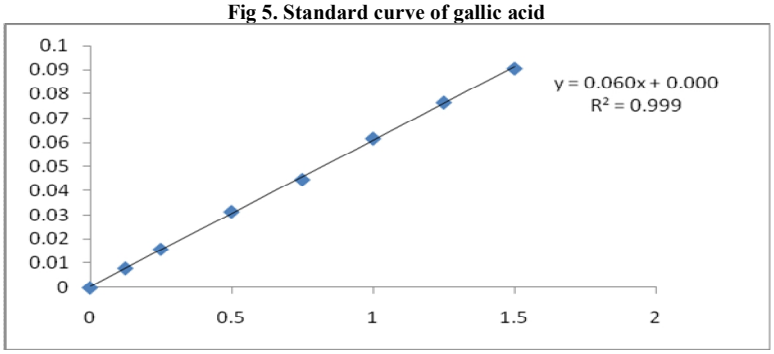

Fig 6. Standard curve of quercetin

Table 3: Comparison of flavonoid content, flavonol content, phenolic and polyphenolic content and proanthocyanidine content obtained from seeds of *D. fastuosa* Linn.

D. fastuosa seed extract	Flavonoid content (mg/g)	Flavonol content (mg/g)	Phenolic and polyphenolic content (mg/g)	Proanthocyanidine content (mg/g)
Methanolic	6.34	5.37	9.97	1.42
Hydroalcoholic	9.35	5.36	6.88	0.88

Antioxidant Activity

Ten different concentrations of test extracts (methanolic and hydroalcoholic), ranging from 2- 1000 µg/ml were prepared and tested for their anti-oxidant activity in different *in- vitro* models. It was observed that free radicals were scavenged by the tested compounds in a concentration dependent manner up to the given concentration in DPPH and iron chelating models. The half maximal inhibitory concentration (IC_{50}) of methanolic extract of *D. fastuosa* by DPPH assay was found to be 28.34 µg/ml & for hydroalcoholic extract 25.78 µg/ml.

Total Antioxidant Capacity: The antioxidant activity of methanolic and hydroalcoholic extracts of *D. fastuosa* at 2, 4, 8, 16, 32, 64, 128, 226, 512, 1000 µg/ml concentrations was measured by phosphorous-molybdenum method. It was calculated using the standard curve of ascorbic acid (y = 0.007x - 0.1751; R^2 = 0.9811) (Fig.3) and expressed as number of equivalent of ascorbic acid per gram of plant extract. The total antioxidant capacity of methanolic and hydroalcoholic extract was found to be 6.83 mg/g and 7.44 mg/g of plant extract respectively (expressed as ascorbic acid equivalents).

Total flavonoid content: Total flavonoid contents of both methanolic and hydroalcoholic extracts were calculated (mg/g) using the standard curve of gallic acid from equation (y = 0.001x+0.005, R^2 = 0.999, where y was the absorbance and x, concentration as shown in Fig 4. Total flavonoid contents of methanolic and hydroalcoholic extract is shown in Table 3.

Total Phenolic Content: Total phenolic content of both methanolic and hydroalcoholic extracts were calculated (mg/g) using the standard curve of gallic acid from regression equation (y = 0.013x +0.127, R^2 = 0.988) where y was the absorbance and x (concentration) as shown in Fig 5. Total phenolic content of both methanolic and hydroalcoholic extracts is shown in Table 3.

Total flavonol Content: Total flavonol content of both methanolic and hydroalcoholic extracts were calculated (mg/g) using the standard curve of quercetin from regression equation (y = 0.060x +0.000, R^2 = 0.999) where y was the absorbance and x, concentration as shown in Fig 6. Total flavonol content of both methanolic and hydroalcoholic extracts is shown in Table 3.

Total pro-anthocynidine content: Total pro-anthocynidine content of both methanolic and hydroalcoholic extracts were calculated (mg/g) using the standard curve of catechin from equation (y = 0.5825x, R^2 = 0.9277). [20] Total pro-anthocynidine content of both methanolic and hydroalcoholic extracts is shown in Table 3.

CONCLUSION

There has been increasing interest in the development of new types of effective and nontoxic antimicrobial compounds. As the rapid emergence of drug-resistant organisms necessitates the continuous search of new antimicrobial substances, natural products may act as alternative for antibiotics and chemotherapeutic agents in certain circumstances. Based on our studies, *D. fastuosa* seeds showed strong *in vitro* free radical scavenging effect in cell free system. However, phytochemical research is still required to identify the active principles responsible for the biological activity of this medicinal plant.

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