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ORIGINAL RESEARCH ARTICLE

Antibacterial Evaluation and Phytochemical Screening of *Datura metel* Leaf Extracts against Bacterial Pathogens

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ABSTRACT

Infectious disease can become a threat to public health in this world. The use of medicinal plants for the treatment of various diseases is an old practice in most countries and it still offers a enormous potential source of new anti-infective agents. The present study was conducted to evaluate the antibacterial potentiality of ethanol and ethyl acetate solvent extracts of mature leaves of *Datura metel* against nine pathogenic bacterial isolates *viz*., *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Escherichia coli, Salmonella typhi, Shigella flexneri, Klebsiella pneumoniae, Vibrio cholerae* and *Pseudomonas aeruginosa*. The turbidity of the bacterial inoculums was compared with 0.5 Mc Farland standards and the antibacterial potential of *Datura metel* ethanol extract was tested by using Agar well diffusion method. The ethanol extract of *Datura metel* (100 mg/ml) showed maximum zone of inhibition (26 mm) against *Pseudomonas aeruginosa, Escherichia coli* and *Bacillus subtilis*. *Staphylococcus aureus* showed less zone of inhibition (8 mm). The ethyl acetate extract of *Datura metel* (100 mg/ml) showed maximum zone of inhibition (19 mm) against *Escherichia coli*. There was no zone of inhibition against *Pseudomonas aeruginosa*. Phytochemical tests were performed and showed that the antibacterial activity of *Datura metel* plant leaves was due to the presence of phytochemical compounds like alkaloids, tripenoid, steroids, flavonoid, triterpenes, phenolic compounds and tannins.

Key words: Antibacterial activity, Ethanol extract, Ethyl acetate extract, Zone of inhibition, *Datura metel*, Bacteria and Phytochemical analysis.

1. INTRODUCTION

A large portion of the world population, especially in developing countries depends on the traditional system of medicine for a variety of disease. Several hundred genera are used medicinally and plants are vital sources for potent and powerful drugs[1]. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, of the phytochemical constituents found *in vitro* to have antimicrobial properties[2]. Many of the spices and herbs used today have been valued for their antimicrobial effects and medicinal powers in addition their flavor and fragrance qualities [3-5].

In India, 500 medicinal plant species are used to pathogenic bacteria. Plants have been used as traditional medicine since time immemorial to control bacterial [6], viral and fungal disease. Recently, research has been initiated to evaluate the feasibility of using herbal medicines in disease management [7]. Moreover, the bacterial infections are considered the major cause of mortality in aquaculture [8]. Because of the growing bacterial resistance against commercial standard and reserve antibiotics, the search for new active substances with antibacterial activity against pathogenic bacteria is of increasing importance [9, 10]. Recently several reports have carried out with antimicrobial activity against bacteria and fungi. Hence the present study was made an attempt to find out the potential effect of coastal medicinal plants against isolated ornamental fish bacterial and fungal pathogens [11].

Plants have been an essential part of human society since the start of civilization. Around 250 drugs have been identified from plants during Rig Veda and Atharvana Veda descriptions of the Veda period. The rural population in different parts of the world is more disposed to traditional ways of treatment because of very easy availability and cheaper cost. It is estimated that 80% of the black population is consulting with traditional healers [12]. In recent years, drug resistance to human pathogenic bacteria has been commonly and widely reported in literature [13].

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Because of the side effects and the resistance that pathogenic microorganisms built against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines [14]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of world [15,16].

Datura metel Linn *(*Thorn-apple, Devil trumpet, Solanaceae) is a Nigerian medicinal plant widely used in phytomedicine to cure diseases such as asthma, cough, convulsion and insanity [17]. The leaves and seeds are widely used in herbal medicine as anesthetic, antispasmodic, antitussive, bronchodilator and as hallucinogenic [18]. The whole plant particularly the leaves and seeds are used as anesthetic, anodyne, anti-asthmatic, antispasmodic, anti-tussive, bronchodilator, and hallucinogenic [19]. The plant finds application in the treatment of catarrh, diarrhea and skin diseases [20]. It is used in the treatment of catarrh, diarrhea, epilepsy, insanity, hysteria, rheumatic pains, hemorrhoids, painful menstruation, skin ulcers and wounds. It is also used in the treatment of burns. It is used to calm cough and to treat laryngitis and treachitis.

Several studies [21, 22] have documented the scientific basis for the efficacy of plants in phyto-medicine. This study seeks to ascertain the usefulness of *Datura metel* in the treatment of infectious conditions caused by common pathogenic bacteria. The present study has been planned to evaluate the antibacterial activity and phytochemical compounds of *Datura metel* against some pathogenic bacteria for possible development of new drugs for the prevention and treatment of infectious diseases caused by bacterial pathogens.

2. MATERIALS AND METHODS

2.1 Collection and drying of plant materials

Mature leaves of *Datura metel* were collected from Medicinal Garden, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu. The leaves of *Datura metel* were washed thoroughly three times with water and once with distilled water. The plant materials were shade dried and powdered. The powdered samples were hermetically sealed in separate polythene bags.

2.2 Preparation of plant extract

40 g of powdered leaves of *Datura metel* were extracted successively with 200 ml of ethanol at 56-60°C and ethyl acetate at 40-50°C in Soxhelet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use [23].

2.3 Test microorganisms

Nine pathogenic bacteria, *viz*., *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Vibrio cholerae* and *Pseudomonas aeruginosa* were used during the present study and were obtained from Rajah Mutthaiya Medical College Hospital, Chidambaram. The cultures were sub-cultured and maintained on Nutrient agar slants and stored in refrigerator at 4°C.

2.4. Determination of antibacterial activity

2.4.1 Preparation of Mc Farland Nephelometer standards

10 test tubes of equal size and good quality have been thoroughly cleaned and arranged in the test tube stand. 1% chemically pure Sulphuric acid and 1.175% aqueous solution of Barium chloride was prepared. Slowly and with constant agitation, the designated amounts of two solutions were added to the tubes as shown in (**Table 1**) to make a total volume of 10 ml per tube. The tubes were sealed. The suspended Barium chloride precipitate corresponds approximately to homogenous cell densities per ml throughout the range of standards as shown in table. Store the Mc Farland standard tubes in the dark at room temperature. They should stable for 6 months.

Table 1. Mc Farland Nephelometer standard.

Chemical Name / std	0.5	1	2	3	4	5	6	7	8	9	10
Barium chloride (ml)	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
Sulfuric acid (ml)	10	9.9	9.8	9.7	9.6	9.5	9.4	9.3	9.2	9.1	9
Approximate cell density (x 10^8/ml)	1.5	3	6	9	12	15	18	21	24	27	30

2.4.2. Inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc Farland standards.

2.4.3 Determination of antibacterial activity (Agar well Diffusion Method)

The antibacterial activity of *Datura metel* leaf extract was determined by Agar well diffusion method. The Muller Hinton agar (MHA) plates were prepared and inoculated with test organisms by spreading the bacterial inoculum on the surface of the media. Wells (8 mm in diameter) were punched in the Muller Hinton agar plates. Ethanol and ethyl acetate leaf extracts of *Datura metel* with different concentrations (25 mg/ml, 50mg/ml, 75mg/ml and 100 mg/ml) were mixed with 1 ml of Dimethyl sulfoxide (DMSO) and added into the well. Well containing DMSO alone act as a negative control. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (in mm).

2.5 Phytochemical analysis

Phytochemical tests were done to find the presence of the active chemical constituents present in *Datura metel* leaf powder such as alkaloid, glycosides, terpenoids, steroids, flavonoids, reducing sugars, triterpenes, phenolic compounds and tannins by the following procedure.

2.5.1 Test for Alkaloids (Meyer's Test)

The extract of *Datura metel* was evaporated to dryness and the residue was heated on a boiling water bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent (Siddiq and Ali, 1997). The samples were then observed for the presence of turbidity or yellow precipitation [24].

2.5.2 Test for Glycoside

To the solution of the extract in Glacial acetic acid, few drops of Ferric chloride and Concentrated sulphuric acid are added, and observed for reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer [25].

2.5.3 Test for Tripenoid and Steroid

4 mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoid and green bluish colour for steroids [25].

2.5.4 Test for Flavonoid

4 mg of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavones [25].

2.5.5 Test for Reducing sugars

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

2.5.6 Test for Triterpenes

300 mg of extract was mixed with 5 ml of chloroform and warmed at 80°C for 30 minutes. Few drops of concentrated sulphuric acid was added and mixed well and observed for red colour formation.

2.5.7 Test for Phenolic Compounds (Ferric chloride test)

300 mg of extract was diluted in 5 ml of distilled water and filtered. To the filtrate, 5% Ferric chloride was added and observed for dark green colour formation.

2.5.8 Test for Tannins

To 0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution wad added. Blue colour was observed for gallic tannins and green black for catecholic tannins [26].

3. RESULTS AND DISCUSSION

The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant although, it is usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct [27]. The screening of plants usually involves several approach; ethno botanical approach is one of the common methods that are employed in choosing the plant for pharmacological study.

India has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine. A country like India is very much suited for development of drugs from medicinal plant. Because of its vase and wide variations in soil and climate, the Indian sub – continent is suitable for cultivation of large number of medicinal and aromatic plant which can be used as raw materials for pharmaceutical, perfumery, cosmetics, flavour and food and agrochemical industries. A large number of these plants grow wild and exploited especially for use in indigenous pharmaceutical houses. Some of these plants produce valuable drugs which have high export potential [28].

In the modern world multiple drug resistance has developed against many microbial infections due to the indiscriminate use of commercial

antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [29].

In the present study, antibacterial activities of ethanol and ethyl acetate extract of *Acalypha indica* was assayed against various bacterial pathogens and the results were showed in (**Table 2 & 3**). The zone of inhibition of 100mg ethanol extract of *Datura metel* against bacteria was, *Staphylococcus aureus* (8 mm), *Bacillus cereus* (11 mm), *Bacillus subtilis* (26 mm), *Salmonella typhi* (21 mm), *Shigella flexneri* (21 mm), *Escherichia coli* (26 mm), *Klebsiella pneumoniae* (16 mm), *Vibrio cholerae* (16 mm) and *Pseudomonas aeruginosa* (26 mm). The zone of inhibition of 100mg ethyl acetate extract of *Datura metel* against bacteria was, *Staphylococcus aureus* (13 mm), *Bacillus cereus* (9 mm), *Bacillus subtilis* (9 mm), *Salmonella typhi* (14 mm), *Shigella flexneri* (11 mm), *Escherichia coli* (19 mm), *Klebsiella pneumoniae* (16 mm), *Vibrio cholerae* (9 mm) and *Pseudomonas aeruginosa* showed no zone of inhibition. In comparison, the zone of inhibition of ethanol extract of *Datura metel* against bacteria was more when compared to ethyl acetate extract except in *Bacillus cereus*.

Saranraj *et al*., (2010) [30] recently investigated the antibacterial potentiality of ethanol and ethyl acetate extract of *Acalypha indica* leaves against human pathogenic bacteria and concluded that and concluded that the ethanol extract showed more inhibitory activity against human pathogenic bacteria when compared to ethyl acetate extract. The findings of the present study coincide with the results of Saranraj *et al*., (2010) [30]. In this study, ethanol was best solution for extracting the effective antimicrobial substances from the medicinal plant *Datura metel* than ethyl acetate. The ethanol extract *Datura metel* showed effective results against all test organisms but the ethyl acetate extract of *Datura metel* was low effective. This could be related to the presence of bioactive metabolites present in *Datura metel* which are not soluble in ethyl acetate but they can be soluble in ethanol.

Sundaram Ravikumar *et al*., (2010) [31] screened the *in vitro* antibacterial and antifungal activity of the chloroform extracts of the seventeen different coastal medicinal plants against different gram positive and gram negative and fungal ornamental fish pathogens. Of the selected plants *Datura metel* showed wide range of antimicrobial activity against many of the fish pathogens. He concluded that the *Datura metel* has been used as a putative antimicrobial drug in the aquaculture maintenance.

Some studies concerning the effectiveness of extraction methods highlight that methanol extract yields higher antibacterial activity than n-hexane and ethyl acetate [32]. Whereas other report that chloroform is better than methanol and benzene [33]. It is clear that using organic solvents provides a higher efficiency in extracting compounds for antimicrobial activities compared to water based method [34].

John De Britto and Herin Sheeba Gracelin, (2011) [35] investigated the phytochemicals present in leaves, stem, flowers and fruits of *Datura metel* which have some medicinal applications. Phytochemical analysis gave positive results for steroids, triterpinoids, reducing sugars, sugars, alkaloids, phenolic compounds, flavonoids and tannins. The stem and fruits extracts did not show marked antibacterial activity. The phytochemical compounds of *Datura metel* extract were analyzed in the present study and the results were showed in (**Table 4**). It showed the presence of alkaloids, tripenoid, steroids, flavonoid, triterpenes, phenolic compounds and tannins. Antibacterial activity of *Datura metel* was due to the presence of phytochemical compounds.

Table 2: Antibacterial activity of *Datura metel* ethanol extract against bacterial pathogens.

S. No	Organisms	Concentration of extract and zone of inhibition			
		25 mg	50 mg	75 mg	100 mg
1	*Pseudomonas aeruginosa*	NZ	11 mm	21 mm	26 mm
2	*Escherichia coli*	5 mm	14 mm	22 mm	26 mm
3	*Shigella flexneri*	NZ	9 mm	16 mm	21 mm
4	*Staphylococcus aureus*	NZ	NZ	NZ	8 mm
5	*Klebsiella pneumoniae*	NZ	5 mm	8 mm	16 mm
6	*Salmonella typhi*	NZ	12 mm	17 mm	21 mm

7	*Vibrio cholerae*	NZ	NZ	9 mm	16 mm
8	*Bacillus cereus*	NZ	NZ	5 mm	11 mm
9	*Bacillus subtilis*	6 mm	14 mm	22 mm	26 mm

NZ – No zone

Table 3: Antibacterial activity of *Datura metel* ethyl acetate extract against bacterial pathogens.

S. No	Organisms	Concentration of extract and zone of inhibition			
		25 mg	50 mg	75 mg	100 mg
1	*Pseudomonas aeruginosa*	NZ	NZ	NZ	NZ
2	*Escherichia coli*	NZ	9 mm	14 mm	19 mm
3	*Shigella flexneri*	NZ	NZ	7 mm	11 mm
4	*Staphylococcus aureus*	NZ	5 mm	9 mm	13 mm
5	*Klebsiella pneumoniae*	5 mm	11 mm	13 mm	16 mm
6	*Salmonella typhi*	NZ	7 mm	11 mm	14 mm
7	*Vibrio cholerae*	NZ	NZ	NZ	9 mm
8	*Bacillus cereus*	NZ	NZ	NZ	7 mm
9	*Bacillus subtilis*	NZ	NZ	8 mm	9 mm

NZ – No zone

Table 4: Phytochemical analysis of *Datura metel* extracts

S. No	Test	Result
1	Alkaloids	+
2	Glycosides	-
3	Tripenoid and steroid	+
4	Flavonoid	+
5	Reducing sugars	-
6	Triterpenes	+
7	Phenolic compounds	+
8	Tannins	+

4. CONCLUSION

The study of antibacterial activity of herbal plant extract of *Datura metel* showed that the ethanol extract shows promising antibacterial activity against bacterial human pathogens when compared to ethyl acetate extract. Phytochemical analysis showed that the antibacterial activity of *Datura metel* was due to the presence of phytochemical compounds like alkaloids, tripenoid, steroids, flavonoid, triterpenes, phenolic compounds and tannins. The results also indicated that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results. These plants could serve as useful source of new antimicrobial agents.

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