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PHYTOCHEMICAL SCREENING OF *DATURA METEL LINN* AND ITS ANTIMICROBIAL ACTIVITY ON SELECTED HUMAN PATHOGENS

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Abstract: Medicinal plants are a source of great economic value all over the world. Various medicinal plants have been used for years in daily life to treat diseases. Present study illustrates the phytochemical screening and antimicrobial activity of *Datura metel.* The selected plant leaves were collected and powdered. The bioactive compounds were extracted by using acetone, chloroform and water in a soxhelet extractor. The antimicrobial activity was determined by using agar disc and well diffusion method. Acetone and chloroform extracts were mixed with 1ml dimethyl sulfoxide (DMSO) and added into the well. The extract of acetone with *Datura metel* has shown maximum zone of inhibition against bacterial pathogens when compared to chloroform and water extract. Phytochemical analysis reflected the antimicrobial activity of *Datura metel* which is due to the presence of phytochemical compounds like alkaloids, terpenoids, steroids, flavonoids, saponins, phenolic compounds and tannins. The results of agar well diffusion method indicated the inhibition and it depends largely upon plant parts used and organism tested. The present study showed good inhibitory activity of acetone extracts than the chloroform and water extracts. In future studies *Datura metel* will be tested in the treatment of infectious diseases caused by resistant pathogenic microorganisms.

Key Words: Antimicrobial activity, *Datura metel*, Extract, Phytochemical and Resistance.

INTRODUCTION

Nature has been showed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Medicinal plants are employed for the ailment of several microbial and non-microbial diseases due to their valuable effects in health care. The affordability, reliability, availability and low toxicity of medicinal plants in therapeutics made them popular and acceptable by all religions and implementation in health care all over the world (Akharaiyi, 2011). Plants have been producing a diverse range of bioactive molecules, making them rich sources of different types of medicines. Higher plants, sources of medicinal compounds have continued to play a dominant role in maintenance of human health since ancient times (Farombi, 2003). Over 50% of all modern clinical drugs are of natural origin. Natural products play an important role in drug development programs in the pharmaceutical industry (Baker *et al.*, 1995).

Datura leaves are used for herbal medicine as anesthetic, antispasmodic, bronchodilator and hallucinogenic (Dabur *et al.*, 2004). *Datura metel Linn* also called as Indian Thorn Apple belongs to the family solanaceae which consists of 85 genera and 2500 species worldwide. *Datura metel* Linn is a perennial herbaceous plant and can reach the height of 1.5m (Figure 1). Leaves are simple alternate, dark green, broadly ovate, shallowly lobed and glabrous. Flowers are large, solitary and trumpet-shaped with a sweet fragrance. The fruit is in the form of capsule with short spine covering. *Datura* is one of the most interesting plants with hallucinogenic properties. The whole plant is antiseptic, narcotic, sedative and is useful for asthma (Moghadam *et al.*, 2010). It is also used in the treatment of burns, calm cough and to treat laryngitis and treachitis (Prasanna and Yuwvaranni, 2014).Despite having a reputation as one of the darker hallucinogens, it has been widely used by society historically in both the old world and the new, which continues today for those interested in ethanobotanical uses of this plant worldwide. The demand of alkaloid content in the past posed its application as a subject for botanical research is vast nowadays. It is a genus of contrasts from smelly weeds to lovely ornamentals (Jamdhade *et al.*, 2010). A variety of phyto chemicals have been found to occur in *Datura metel*. These phytoconstituents comprises alkaloids, flavonoids, phenols, tanins, saponins and sterols. The solanaceous alkaloids hyoscyamine, scopolamines have been isolated from *Datura metel* (Chopra *et al.*, 1986).

Figure 1: A view showing leaves and flowers of *Datura metel*

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The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing at the present time. Plant extracts have greater potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious disease caused by pathogens. A number of reports have been carried out to focus the antimicrobial activity against bacteria and bacterial pathogens (Okoye *et al.*, 2010). Many plants have therapeutic and pharmaceutical effects such as antimicrobial, antioxidant, anti-infectious and anti-tumor activities (Akroum *et al.*, 2009). Application or drinking of leaf juice relieves pain and swelling. Reduction of breast pain is noticed when leaf juice mixed with lime and turmeric are applied to painful area (Rahmatullah *et al.*, 2010).

According to WHO the best source of medicines are medicinal plants, therefore such plant should be studied and evaluated properly to check there structural and functional properties as well as the particular activity of each parts of the plants (Manjamalai *et al.*, 2010) In the present study preliminary study of antibacterial activity as well as phytochemical screening has carried out against the selected pathogens viz *Bacillus subtilis, Salmonella typhi* and *E. coli.* Phytochemical activities on the leaves of *Datura metel Linn* were tested and evaluated against the resistance pathogens inhabit in human body.

MATERIAL AND METHODS

Collection and Preparation of plant materials

The fresh and disease free leaves of *Datura metel* were collected from agricultural form house at vennandhur, Namakkal district. The fresh leaves were washed with distilled water, shade dried and the leaves were completely powdered by the use of grinder.

Preparation of extracts

The air dried and powdered plant materials [1000mg for each] were extracted with 10ml of acetone, chloroform and water by using soxhelet apparatus for 72 hours at a temperature not exceeding the boiling point. The powdered sample was added to sohxlet apparatus and solvent is added to it in 1:5 ratios. The solvent system used in as an increasing order of polarity [acetone-Chloroform-Distilled water]. Then the extract was collected from sohxlet apparatus and kept in room temperature for air-drying. The residues were collected, weighed and stored in 4˚C for future use (Harbone, 1998).

Phytochemical Analysis

Phytochemical tests were done to find out the presence of bioactive chemical constituents such as alkaloids, terpenoids, flavonoids, carbohydrates, tannins, saponins and steroids - amino acid compounds by the following procedure.

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 (Harbone, 1998).

Test for Terpenoids: 4mg of extract was treated with 0.5ml 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 (Siddiq and Ali, 1997).

Test for Flavanoids: 4mg of extract solution was treated with 1.5ml 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 flavanoids and orange colour for flavones (Harbone, 1998).

Test for Carbohydrates: Treat the test solution with few drops of alcoholic alpha-napthol. Add 0.2ml of Concentrated Sulphuric acid slowly through the sides of the test tube, a purple to violet color ring appears at the junction (saranraj *et al.*, 2011).

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 (Anpin Raja *et al.*, 2010).

Test for Saponins: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously. Persistent froth indicated the presence of saponins (saranraj *et a*l., 2011).

Test for Steroids: 4 mg of extract was treated with 0.5ml of acetic anhydride and 0.5ml 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 (Siddiq and Ali, 1997).

Antibacterial Activity

Test organisms: Three pathogenic bacteria viz., *Bacillus subtilis, Salmonella typhi*, and *E.coli* were collected from Selvamm Arts and Science College, Namakkal. The cultures were subcultured and

maintained on Nutrient agar slants in refrigerator at 4°C.

Inoculum preparation: Stock Cultures were maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of culture from the stock to test tubes of nutrient broth for bacteria and incubating for 24 hours at 37 °C and 25°C respectively. The cultures were diluted with fresh nutrient broth.

Agar well diffusion method: The antibacterial screening of the crude extracts were evaluvated by agar well diffusion method. After solidification of the medium, a well was made in the plates with sterile borer [5mm]. The extract compound 70, 80, 90µl was introduced into the well and the plates were incubated at 37°C for 24 hours. All samples were tested in triplicates. The microbial growth was determined by measuring the diameter of the zone of inhibition.

Agar disc diffusion method: The antibacterial activity of different extracts of leaves of Datura metel was tested in disc diffusion method. The disc containing test samples were placed on agar medium seeded with selected microorganisms. Standard antibiotic discs and blank discs were used as positive and negative control. The plates were then incubated at 37°C for 24h to allow maximum growth of the microorganisms. The antibacterial activity of the test samples were determined by measuring diameter of the inhibition zone expressed in millimeter (Koochak *et al.*, 2010).

RESULTS AND DISCUSSION

The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant even it is not usually 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 (Agrawal *et al.*, 1996). 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. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of world (Aqib Sayyed and Mohib Shah, 2014).

The phytochemical screening of *Datura metel* revealed the presence of important pharmacological bioactive substances as well as medicinal and nutritional potentials in the leaves. The antibacterial activities of the studied plant extracts were comparable to the reference antibiotic (positive control) used. Therefore, this study offers a scientific basis for the use of the plant extracts for the treatment of infections that could be caused by the strains of the test bacterial organisms. It is thus suggested that more studies on concentrations of active ingredients, antinutritional factors and toxicity level be carried out. From the plant saponins, flavonoids, phenols, alkaloids, glycosides, and steroids were identified in aqueous extract (Akharaiyi, 2011). In the chloroform extract steroids, flavonoids, triterpenoids were absent and tannins was absent in the acetone extract (Irudayaraj *et al.*, 2010). Current study agrees the above literature except tanins which was not recorded in any of the extracts used.

Table 1: Phytochemical Analysis of *Datura Metel*

S.No	Phytochemical Compounds	Acetone	Chloroform	Aqueous
1.	Steroids	+	-	+
2.	Alkaloids	+	+	+
3.	Flavanoids	+	+	+
4.	Triterpenoids	+	-	+
5.	Saponins	+	+	+
6.	Tannins	-	-	-
7.	Phenols	+	+	+
8.	Glycosides	+	+	+

The results of present study showed that the selected plant *Datura metel* extracts were effective against the bacterial species tested. This can be used to treat *Bacillus subtilis, Salmonella typhi* and *E. coli.* Table 1 highlights the phytochemical yield of the plant aqueous and other extracts. However, the aqueous extracts contained more of the identified phytochemical compounds than the other two extracts. Majority of the phytochemical compound identified in the aqueous extract have been reported distinctly with therapeutic importance. It is observed that the leaf content has more phtochemicals as compared to other plant parts where as it is very less in stem (Jamdhade *et al.*, 2010). Current study agrees that the leaf extracts of *Datura metel* inhibited the tested bacterial isolates. The higher inhibition on the bacterial species by acetone extract of the leaves (24mm), chloroform leaf extract (23mm) and aqueous leaf extract (22mm) (Suresh and Nagarajan, 2010).

According to Prasanna and Raghunathan (2014), in most cases the methanol and ethanol extracts exhibited higher antibacterial effects than the corresponding extracts. Impregnated paper discs containing only DMSO used as negative control did not show any inhibition zone. Since the size of the zone of inhibition depends upon both the rate of diffusion of the active agent into the plate and of the rate of growth of the target microorganism, the sizes of the inhibition zone can only be interpreted as an indication of microbial susceptibility or resistance in a clinical

setting with well characterised antibiotics (Veni and pushpanathan, 2014). The inhibition activity of plant extracts against the growth of microorganisms was attributed to the presence of antioxidants (Arshad *et al.*, 2011).

Table 2: Agar Disc Diffusion Method

Extracts 10mg / ml		Microorganisms (mm)		
		B. subtilis	*E. coli*	*S. typhi*
Acetone	70	4	5	7
	80	7	10	10
	90	9	13	15
	100	12	17	19
Chloroform	70	5	4	5
	80	7	6	8
	90	10	9	12
	100	14	13	17
Aqueous	70	5	6	5
	80	8	10	7
	90	14	13	10
	100	17	19	16

Table 3: Agar Well Diffusion Method

Extracts 10mg / ml		Microorganisms (mm)		
		Bacillus subtilis	*E.Coli*	*Salmonella typhi*
Acetone	70	8	6	7
	80	11	13	14
	90	18	16	18
	100	20	24	20
Chloroform	70	6	9	10
	80	10	12	15
	90	15	18	19
	100	23	21	23
Aqueous	70	5	10	8
	80	12	14	13
	90	16	17	19
	100	20	22	22

CONCLUSION

The present antimicrobial of different crude extracts of *Datura metel* showed that the chloroform, acetone, and aqueous from dry leaves shows highest zone formation in acetone extract activity against the employed bacteria. Similarly the chloroform and aqueous extracts showed the lowest activity of antimicrobial activities of the crude extracts of *Datura metel* depends on the presence of phytochemicals such as alkaloids, steroids, flavonoids, tripenoids, saponins, and glycosides. Tannins where absent in aqueous and acetone extracts. This plant crude extracts could serve as potential sources of antimicrobial agents. Further research is needed towards isolation and identification of active principles present in the extracts which could be used for pharmaceutical use.

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