IN-VITRO EVALUATION OF DATURA METEL LEAF FOR POTENTIAL ANTIMICROBIAL ACTIVITY AGAINST WOUND CAUSING PATHOGEN

Manikandan.R and Ananth. A

Department of Microbiology, Srinivasan College of Arts & Science, Perambalur, Tamilnadu State, India.

ABSTRACT

Datura metel leaves has been used as a medicine for the treatment of various diseases including wound healing. Pharmacognostical and phytochemical parameters determined in the present work can serve as major criteria for identity, quality and purity of a crude drug and extracts. Antibacterial activity of the plant extracts show different inhibition spectrum against the isolated wound causing bacterial pathogens. Among them leaf ethanol extract shows good antibacterial activity when compared to aqueous extracts. Results of antimicrobial activity revealed that the extracts showed good antimicrobial activity against all pathogens tested especially *Pseudomonas sp, Klebsiella sp.* and *Bacillus sp*. On the basis of the phytochemical assay results it is concluded that the extracts contain higher quantities of alkaloids compounds present. Phytochemical screening of leaf ethanol extract of *Datura metel* L showed good positive results for major phytochemical constituents namely Alkaloids, tannins, saponins, carbohydrates, alkaloids, protein and aminoacids when compared to aqueous extracts. Hence, this study provided a good medicinal plant based treatment strategy and will create social awareness among the wound healing.

Keywords: *Datura metel*; Phytochemicals; Alkaloids.

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 affordability, reliability, availability and low toxicity of medicinal plants in therapeutic use has made them popular and acceptable by all religions for implementation in medical health care all over the world. Plants are indeed the first material used in alternative medicine type of remedy against many diseases.

Several plants have therapeutic and pharmaceutical effects, for antimicrobial, antioxidant, anti-infectious and antitumour activities (Akroum & Akroum).

Wounds are inescapable events of life, which arise due to physical injury, chemical injury or microbial infections. Healing of wounds usually takes place in a direction away from its normal course and under healing, over healing or no healing of wounds is common. Wound is defined simply as the disruption of the cellular and anatomic continuity of a tissue. The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue. Clinically, one often encounters non-healing, under-healing or over healing (Bennet, 1988).

Datura metel Linn. has been extensively used for various medicinal purposes throughout the world. The plant is widely used in traditional medicine to cure diseases such as asthma, cough, wound treatment, convulsion, headache and insanity (Duke and Ayensu **1985)**.The leaves and seeds are used as anesthetic, antispasmodic, bronchodilator, hallucinogenic and myristic medicines (Barefoot, **1992**). *D. metel* has been extensively used in traditional medicine to cure some ailments such as epilepsy, insanity, asthma, cough, wounds, burns, hemorrhoids, rheumatism and painful menstruation. These functions are due to many bioactive compounds available in the plant (Nadkarni, 1976).

Datura metel is a shrub-like perennial herb, commonly known as angel's trumpet, devil's trumpet, and metel, commonly in the solanaceae family. *Datura metel* grows in the wild in all the warmer parts of the world, and is cultivated worldwide for its chemical and ornamental properties. It is not possible to be sure about its original home (Linnaeus, 1753). All parts of *Datura* plants contain dangerous levels of tropane alkaloids (highly poisonous) and may be fatal if ingested by humans or other animals, including livestock and pets (Hans-Georg Preissel , 2002).

Therefore the aim of treating a wound is to either shorten the time required for healing or to minimize the undesired consequences. Attention should be directed towards discovering an agent, which will accelerate wound healing either when it is progressing normally, or when it is

suppressed by various agents of *Datura metel L* against the pathogens and also screened for its pharmocognostic and phytochemical features.

MATERIALS AND METHODS

Collection of wound pus samples:

Pus sample was collected in a wide mouthed container (Hi-media) from clinically diagnosed patients. After collection of samples, the containers were closed tightly to avoid any leakage during transportation. The sample was not refrigerated, as some of the enteric pathogens are highly sensitive to temperature. While collecting the sample, care was taken to label the sample. The label had the name of the patient, date of collection, age and sex of the patients and clinical condition along with hospital name, ward name; ward number and physicians name. In the laboratory, the specimens were registered and swabs were cultured on nutrient broth and incubated at 37ºC for 24 h.

Isolation and identification of wound bacterial isolates:

Culture plates of Eosin methylene blue agar, MacConkey agar, Nutrient agar, Cetrimide agar and Mannitol salt agar (Hi Media, India) were used. The swab sticks used for the collection of the samples were streaked directly on the labeled agar plates and incubated at 37°C for 24 h. After incubation, cultures were examined for significant growth. Subcultures were then made into plates of nutrient agar and incubated for another 24 h. The primary identification of the bacterial isolates was made based on colonial appearance and pigmentation. Biochemical tests were performed to identify the isolates. Biochemical tests applied were standard catalase test, citrate utilization, coagulase, oxidase, methyl red, Voges-Proskauer, indole production, motility, carbohydrate fermentation test using glucose, sucrose, maltose and lactose. Characterization and identification of the isolates was done using the methods of Cowan and Steel (1985), Cheesbrough (2004), Mathur *et al.* (2006) and Senthilkumar *et al.* (2012).

Collection and Drying of plant materials

Mature leaves of *Datura metel* were collected from the near ponds, river bridge and local area of Perambalur district, Tamil Nadu, India. The leaves of D. *metel* were washed thoroughly three times with water and once with distilled water.

Drying and Size Reduction of Plant:

The whole plant material of *Datura metel* (leaves) was subjected to shade drying for about 10 weeks. The dried plant material was further crushed to powder and the powder was passed through the mesh 60 and the powdered samples were hermetically sealed in separate polythene bags until the time of extraction and further analysis.

Determination of Fluorescence Character:

Fluorescence characters of powdered plant material with different chemical reagents were determined under ordinary and ultraviolet light (Chase and Pratt, 1949).

Determination of Physicochemical Parameters:

The dried plant material was subjected for determination of physicochemical parameters such as total ash value, acid insoluble ash value, water soluble ash value, moisture content, foreign organic matter, crude fibre (Raghunathan, 1976), alcohol soluble extractive and water soluble extractive (Usha and Sharma, 1984).

Extraction of Powdered Plant Material:

40 g of powdered leaves *D. metel* were extracted successively with 200 ml of ethanol and methanol at 56-60°C hexane 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 (Chessbrough, 2000).

Preliminary Photochemical Analysis:

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

Test for Alkaloids (Meyer's Test)

The extract of *D. metel* leaves 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 (Evans, 2002).

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 (Siddiq and Ali, 1997).

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 (Siddiq and Ali, 1997).

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 (Siddiq and Ali, 1997).

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.

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.

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.

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 (Iyengar, 1995).

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. 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 (Parekh *et al*., 2005).

Test organisms required for the antimicrobial activity screening were isolated from various samples. Isolated organisms were identified by making use of colony morphology on nutrient agar, colony morphology on selective cum differential agar, microscopic methods and Biochemical methods. Based on these tests the organisms were identified as *Pseudomonas, Klebsiella, and Bacillus.* These organisms involved causes of wound infection in human beings and this infection control of these organisms through herbal way will be more helpful to human society because most of the current antibiotics are toxic to human cells and tissues some of the organism developed resistance against various current antibiotics used.

Fluorescence analysis of day and UV light, plant provides proper authenticity for the quality raw material. *Datura metel* leaves was collected from perambalur district and powdered using mechanical blender. Resulted power showed light green, brown, green coloure and reddish brown etc., (Table-1).

Physiochemical data of the plant powder also provide specific nature of the *Datura metel* leaves plant powder. Results of physiochemical parameter revealed that the plant powder did not have any of the foreign matter. Ash content of the powder material is directly proportional to the

quantity of foreign matter present in the given sample. Total ash value, acid insoluble ash value, of water soluble ash value were within the limit of ayurvedic pharacopeia of India. *D.metel* leaves exhibited 5.4 ± 0.85% total ash, 32.2 ± 0.54% water soluble ash and 14.2 ± 0.46% acid soluble ash (Table -2).

Navaratnarajah and Sashikesh (2011) were reported that moisture content of *Datura metel* was 76.69±0.12. The carbon content as a measure of ash was 6.62±0.23. The mean organic matter was 11.61±0.09. Calcium, magnesium, iron and phosphate contents were (4.28±0.05) $\times 10^4$, (3.86±0.009)$\times 10^4$, (2.33±0.007)$\times 10^4$ and (4.65±0.06)$\times 10^4$ppm respectively.

Table (1) - Fluorescence analysis of drug powder:

S.No	Treatment	Datura	
		Day light	**UV light**
1	Drug Powder	Light Green	Light Green
2	Drug powder + aq.1N NaOH	Reddish browm	Dark Brown
3	Drug powder + alc.1N NaOH	Brown	Dark Brown
4	Drug powder + 1N HCl	Green	Green
5	Drug powder + 50% H_2SO_4	Brown	Dark green

Table (2) - Physico chemical constants:

S. No	Parameters	Value % W/W
1.	Foreign Matter	1.6 ± 0.09
2.	Loss on drying	2.3 ± 0.28
3.	Total Ash Content	5.4 ± 0.85
4.	Water soluble Ash	32.2 ± 0.54
5.	Acid insoluble Ash	14.2± 0.46

Where n=3 (The experiments were repeated for 3times)

Ethanol extract inhibited all pathogens and other organisms in a dose dependent manner. Zone of inhibition produced by ethanol extract of *Datura metel* leaves ranges from 12mm to 26mm. Best activity was noted against *Pseudomonas* (26mm) at 1600µg/disc concerntration. Ethanol extract showed best results when compared to aqueous extract (Table 3).

Kawther Abeb, (2007) evaluated the antibacterial activity of *Datura metel* against *Mycobacterium* sp, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. The results showed that the *Datura metel* extracted by steam distillation method from roots, stem and leaves of plants did not show antibacterial or anticandidal activities.

Table(3) - Antibacterial Activity of *Datura metel Ethanol* Extract

S. No	**Strains used**	**Positive control (mm)**	**Negative control (mm)**	**Extract/concentration (µg/disc) Zone of inhibition in mm**			
				100	**200**	**300**	**400**
1.	*Klebsiella pneumoniae*	11	0	16	17	20	23
2.	*Bacillus cereus*	11	0	12	14	16	17
3.	*Pseudomonas aeruginosa*	11	0	16	19	22	26

John De Britto and Herin Sheeba Gracelin, (2011) investigated the phytochemicals present in leaves, stem, flowers and fruits of *Datura stramonium* 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-3. The phytochemical analysis of *Datura metel* showed the presence of alkaloids, steroids, terpenoids, phenolic compounds and saponins.

Table (4) - Behaviour of Drug powder with various chemical reagents

S.No	Test for	Observation
1	Saponin	Present
2	Tannin	Absent
3	Sterol	Present
4	Terpene	Present
5	Flavonoid	Absent
6	Aminoacids	Present
7	Reducing sugars	Present
8	Lignin	Absent
9	Alkaloid	Present

CONCLUSION

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. The present study justifies the claimed uses of leaves of *D.metel* in the traditional system of medicine to treat various wound infection caused by the microbes on the basis of its reported chemical profile. This study also encourages cultivation of the highly valuable plant in large scale to increase the economic status of the cultivators in the country. The obtained results may provide a support to use of the plant in traditional medicine. Hence, this study provided a good medicinal plant based treatment strategy and will create social awareness among the wound healing.

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BIBLIOGRAPHY

Akroum S., Satta, D. and Lalauoui, K. 2009. Antibacterial, antioxidant, cytotoxic activities and phytochemical screening of some Algerian plants, Euro. J. Sci. Res, 31(2), 289-295.

Bennet, RG (1988). Fundamentals of cutaneous surgery. St. Louis: C.V.Mosby; 778.

C K., Dhia, S.K. and Abeer, K. 2005. 'Genetic relationship among salvia species and antimicrobial activity of their crude extracts against pathogenic bacteria'. Asian Jour, Plant Sci, 4(5), 544-549.

Chang SS, Wu ML, Deng JF, Lee CC, Chin TF, Liao SJ. 1999. Poisoning by Datura leaves used as edible wild vegetables. Vet Hum Toxicol. 41:242-5.

Chase, C.R. and R.J. Pratt, 1949. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J. Amer. Pharm. Assoc., 38: 324-331.

Checke P, Shull L. 1985. Natural toxicants in feeds and poisonous plants. Westport: AVI Publishing Company.

Chessbrough, M. 2000. Medical laboratory manual for Tropical countries, Linacre House, Jordan Hill, Oxford.

Cowan, S.T. and Steel, K.J. (1985). Manual for the identification of medical bacteria (4th Ed.). Cambridge University Press. London. p.217.

Duke J. 1992a. Handbook of Phytochemical Constituents of GRAS Herbs and other Economic Plants. Boca Raton: CRC Press.

Duke J. 1985. CRC Handbook of medicinal herbs. Boca Raton: CRC Press, Inc.

Duke J. 1992b. Handbook of biologically active phytochemicals and their activities. Boca Raton: CRC Press.

Evans, W.C. 2002. Trease and Evan's Pharmacognosy. 5th edition, Haarcourt Brace and Company: 336.

Friedman M, Levin C. 1989. Composition of Jimson weed (*Datura stramonium*) Seeds. Agric. Food Chem., 37: 998-1005.

Hussein K. 1985. Medicinal Plants in Libya. Tripoli: Arab Encyclopedia House.

Iyengar, M.A. 1995. Study of drugs. 8th edition, Manipal Power Press, Manipal, India: 2.

John De Britto and Herin Sheeba Gracelin. 2011. *Datura metel* – a plant with potential as antibacterial agent. *International Journal of Applied Biology and Pharmaceutical technology*, 2 (2): 429-433.

Kawther F. Abed. (2007). Antimicrobial Activity of Essential Oils of some Medicinal Plants from Saudi Arabia. *Saudi Journal of Biological Sciences*, **14 (1)**: 53-60.

Kulkarni, S.K., 2005. Hand book of experimental pharmacology, 3 edition, Vallabh Prakashan, pp: 38.

Kurzbaum A, Simsolo C, Kvasha L, Blum A. 2001. Toxic delirium due to Datura stramonium. Isr Med Assoc J 3:538-9.

Mathur, T., Singhal, S., Khan, S., Upadhyay, D.J., Fatma, T. and Rattan, A. (2006). Detection of bio-film formation among the clinical isolates of *Staphylococci*: An evaluation of three different screening methods. *Ind.J. Med. Microbiol.* 24(1): 25-29.

Navaratnarajah Kuganathan and Sashikesh Ganeshalingam. 2011. Chemical Analysis of *Datura Metel* Leaves and Investigation of the Acute Toxicity on Grasshoppers and Red Ants. *E-Journal of Chemistry*. 8(1):107-112.

Parekh, J., D. Jadeja , S. Chanda. 2005. Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. *Turkey Journal of Biology*, 29: 203-210.

Pla A, Alonso E, Batista-Viera F, Franco L. 2003. Screening for Carbohydrate-binding Proteins in Extracts of Uruguayan Plants. Braz. J. Med. Biol. Res., 36: 851-860.

Raghunathan, 1976. Pharmacopoeia standards for Ayurvedic formulations. Central Council for Research in Indian Medicine and Homeopathy, E-25, Defence Colony, New Delhi-100321.

Russel A, Hardin J, Grand L, Fraser A. 1997. Poisonous plants of North Carolina. Charlotte: North Carolina State University.

Senthilkumar, B., Senthilkumar, N. and Gurusubramanium, G. (2012). Practical Microbiology-A laboratory manual. Panima Publishing Corporation, New Delhi, India (in press).

Siddiqui, A.A., and Ali, M. 1997. Practical pharmaceutical chemistry. First edition, CBS Publishers and distributors, New Delhi: 126-131.

Siva Sakthi,S., P.Saranraj and M.Geetha. 2011. Antibacterial evaluation and phytochemical screening of *Datura metel* leaf extracts against bacterial pathogens. *International Journal of Pharmaceutical and Biological Archives*, 2 (4): 1063-1069.

Stace, Clive. 1997. New Flora of the British Isles. Cambridge University Press. p. 532

Usha, S., J. Pannine and H.P Sharma, 1984. Pharmacognostic studies on *Artemisia scoparia* Waldst and Kit proc. Indian Acad. Sci. (Plantscience), 93: 151-164.