ANTIMICROBIAL ACTIVITY AND ANTIMICROBIAL CREAM PREPARATION FROM CRUDE EXTRACT OF *DATURA INNOXIA* LEAVES

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Abstract

Medicinal plants have a significant role in developing new drugs. The phytochemical tests viz. tests for alkaloids, flavonoids, saponins, tannins, phenol and terpenoids etc. which determines the proximate composition of leaves of *D. innoxia*. Previous studies states that the leaves of *D. innoxia* showed presence of phytochemical compounds, are responsible for the inhibiting the growth of pathogenic microorganisms. The present study is about to investigate antimicrobial effect of crude extract from leaves of *D. innoxia* against some selective pathogenic bacteria and fungi. The antibacterial activity was checked against *E.coli*, *Staphylococcus*, *Pseudomonas*, *Klebsiella*, *S. typhi* and antifungal activity was checked against *Candida* and *Aspergillus niger* by agar well diffusion method. The antibacterial and antifungal effect shows positive results i. e, zone of inhibition against selective bacterial and fungal species. The results showed leaves of *D. innoxia* are effective against selective bacterial and fungal species. Hence it is effective to prepare antimicrobial cream; the antimicrobial cream was prepared using crude extract from leaves of *D. innoxia* by mixing specific ingredients to design new drug and to protect from infectious microbes.

Keywords: *Datura innoxia*, antimicrobial activity, agar well diffusion method, antimicrobial cream.

Introduction

In modern and traditional medicines, medicinal plants constitute an effective source. Plants act as a biosynthetic laboratory for its chemical compounds like alkaloids, phenolics, terpenoids and glycosides. Natural products have played an important role in treatment and prevention of human diseases (Newman *et al.,* 2000; Chin *et al.,* 2006; Kaushik *et al.,* 2008). About 80% of population in developing countries uses medicinal plants as traditional health remedies as they lack access to pharmaceutical drugs (Magrani *et al.,* 2005). Medicinal plants play a significant role in providing primary health care services to rural people and are used by about 80% of the marginal communities around the world (Prajapati and Prajapati, 2002; Latif *et al.*, 2003; Shinwari *et al*., 2006).

The novel drugs were developed by the extraction of biologically active compounds from plants which were screened on the basis of medicinal uses or bioactivity (Hunter, 2001). Plants belonging to family *Solanaceae* are distributed worldwide, which includes 85 genera and about 2,800 species in the world. There are approximately 25 different species of Datura throughout the world, they are often called as Jimson weed or 'Thornapple'. The name Datura is derived from the early Sanskrit Dustura (Mann, 1996) or dahatura. Datura has numerous common names like deadly nightshade, Thorn apple, Stink weed, Jimson weed, Devils apple and angels trumpet (Heiser, 1969).

Compared to edible plants, the chemical composition of *Datura innoxia* has been poorly investigated and most of the available information only deals with traditional and medicinal aspects (Ayuba *et al.*, 2010). Many researchers have inspected long-established uses of medicinal plants, but only a few studies have confirmed antimicrobial properties of these plants (Bhattarai *et al.*, 2008; Shakya *et al.*, 2008). Typical height of this plant is from 0.6 to 1.5 meters. The leaves and stems are protected by soft and short grayish hairs, thus whole plant appears grayish. The trumpet-shaped, white flowers are 12-19 cm long. The fruit of Datura plant is an egg- shaped spiny capsule which is about 5 cm in diameter. Datura seeds have shown 90 % germination rate even after 39 years of storage (Heiser, 1969).

The entire *D. innoxia* plant is rich in tropane, alkaloids particularly scopolamine and hyoscyamine. Some plants produce significantly more scolamine than others. The effects of *D. innoxia* are dependent on dosage and method of preparation. The American Indians say that mild dosage produces medicinal, healing effect, a moderate dosage produces aphrodisiac effects and high dosage produce shamanic visions (Ratsch and Christian, 1998).The whole plant is antiseptic, narcotic, sedative and is useful for asthma (Bhattacharjee and Supriya Kumar, 1998). In many parts of the world, the leaves of *D. innoxia* have been smokes alone or in blends as a most effective treatment for asthama (Ratsch and Christian, 1998). In Israel, a decotion of the leaves is consumed to treat diarrhea and apaste of the leaves is applied externally to treat pain (Dafni and Yaniz, 1994). 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. In earlier study, medicinal plants have been reported to be very beneficial in wound care, promoting the rate of wound healing with minimal pain, discomfort and scarring of the patient (Odimegwu *et al.*, 2008). Creams are semisolid dosage forms intended mainly for external use and commonly consist of two immiscible phases, an oily internal phase and an aqueous external phase, Due to emulsified nature of skin surface, drugs formulated as cream more effectively interact with skin and more readily penetrate through biological membranes (Handali *et al.*, 2011).

The present study is about to check the antimicrobial activities of crude extract from leaves of *D. innoxia* against some selective pathogenic bacteria and fungi were investigated to design new drugs. Therefore, the objective of the present study was to investigate the antimicrobial activity of methanolic

extract of leaves of *D. innoxia* and to prepare antimicrobial cream for the prevention from infectious diseases caused by bacterial pathogens.

Materials and methods

Collection of plant material

The fresh leaves of *D.innoxia* were collected during winter season from natural population of Solapur district, Maharashtra. The sample was packed instantly in polythene bag to avoid decomposition of some bioactive compounds. The leaves were identified with reference to Department of Botany, Walchand College of Arts and Science, Solapur (Tahiya *et al*, 2013).

Preparation of sample

The leaves were washed carefully with water to remove dust particles and dried in shade. Then these leaves were kept in dark condition at 37◦ C for 15-20 days for natural drying. After drying, the powder prepared from leaf sample by using grinder (Firasat *et al*., 2016).

Preparation of extract

The extract was prepared by using maceration technique. The 10 gm of dry leaf powder of *D. innoxia* mixed with 200 ml of methanol solvent in a closed glass container and kept at dark condition for 48 hrs. Separate solvent using Whatman No.1 filter paper (Regasini *et al.,* 2008). Then the solvent was kept for evaporation naturally to obtain semi-solid crude extract. After evaporation this semi-solid extract was collected in a test tube for further study of antimicrobial activity.

Test organisms

The methanol extracts of leaves of *D. innoxia* were tested against bacterial strains viz. *E.coli*, *Staphylococcus, Pseudomonas, Klebsiella, S. typhi* and fungal strain which are *Candida* and *penicillium and Rhizopus*. These following bacterial and fungal strains were obtained from Department of Biotechnology, Walchand College of Arts and Science, Solapur. Bacterial strains were subcultured on a nutrient agar plate and stored at 4◦ C in refrigerator for further process.

Antibacterial activity assay

The antibacterial activity of methanol extract of *D. innoxia* leaves was determined by agar-well diffusion method. The nutrient agar plates were prepared and inoculated with test organisms by spreading the bacterial inoculums on the surface of media. Wells (5 mm in diameter) were punched in the nutrient agar plates. The 50 µl of methanol extract of *D. innoxia* leaves as well as ampicillin and ethanol were added into

the prepared wells. The plates were incubated at 37◦ C for 24 hours. The antibacterial activity was performed by measuring the diameter of zone of inhibition (in mm) (Siva Sakthi *et al.*, 2011).

Antifungal activity assay

The antifungal activity of methanol extract of *D. innoxia* leaves was determined by agar-well diffusion method. The Sabouraud Dextrose Agar (SDA) plates were prepared and inoculated with test organisms by spreading the fungal inoculums on the surface of media. Wells (5 mm in diameter) were punched in the SDA agar plates. The 50 µl of methanol extract of *D. innoxia* leaves as well as ampicillin and ethanol were added into the prepared wells. The plates were incubated at 37◦ C for 24 hours. The antifungal activity was performed by measuring the diameter of zone of inhibition (in mm) (Siva Sakthi *et al*., 2011).

Preparation of antibacterial cream

The compositions and amounts of the formulation ingredients are as shown in (Table 1).

Table 1: Formulation of antibacterial cream

Sr. No.	Ingredients	Quantity (in gm)
1	Stearic acid	2.4
2	Potassium hydroxide	0.135
3	Glycerine	1.05
4	Water (purified)	10

In order to prepare the cream, following ingredients were mixed together as per there quantity, and then 0.3 gm of methanol extract of *D. innoxia* leaves was added (Sharma, 2008).

Results

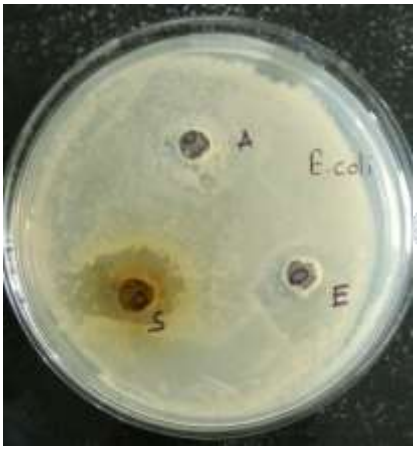
Phytochemical constituents in the plant samples are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal, and anticancer. (Hossain and Nagooru, 2011; Suresh and Nagarajan, 2009).

The antibacterial and antifungal activity of methanol extract of *D. innoxia* leaves against various bacterial as well as fungal strains and the results were showed in (Table 2 and 3) (Fig. 1 and 2).

Table 2: Antibacterial activity of methanol extract of *D. innoxia* leaves against bacterial strains

Sr. No.	Organisms	Zone of inhibition (in mm)

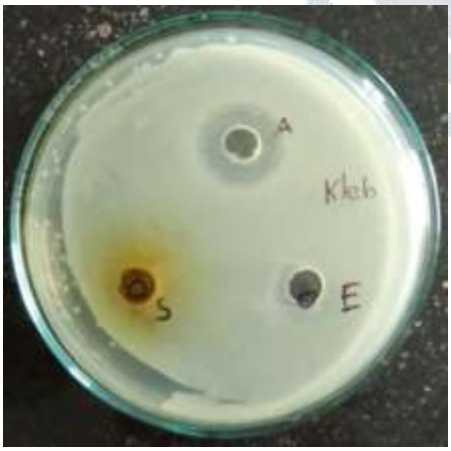
1	*E.coli*	29
2	*Styphalococcus*	31
3	*Pseudomonas*	29
4	*Klebsiella*	31
5	*S. typhi*	30



E.coli

Styphalococcus

Pseudomonas



Klebsiella

S. typhi

Fig.1: Images showing the antibacterial activity of the methanol extract of *D. innoxia* leaves against bacterial strains

Table 3: Antifungal activity of methanol extract of *D. innoxia* leaves against fungal strains

Sr. No.	Organisms	Zone of inhibition (in mm)
1	*Candida*	22
2	*Penicillium*	29
3	*Rhizopus*	21

Candida *Penicillium* *Rhizopus*

The antimicrobial cream were prepared by mixing given ingredients as per there quantity (Fig. 2).

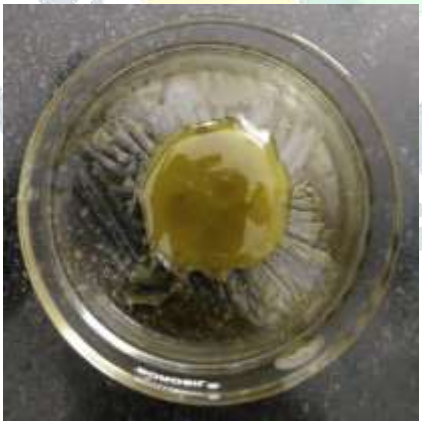


Fig. 2: Antimicrobial cream

Discussion

Plants continue to contribute a myriad of natural products which discover wide range of applications in medicine and other products. In recent years the incidence of multiple resistances in human pathogenic microorganisms has been increasing day by day, mostly because of the indiscriminate use of antimicrobial drugs. The adverse side effects of certain antibiotic and the occurrence of formerly uncommon infections are

a severe problem in medical field (Shito, 2001).The inherited use of plants as a foundation of medicine is a crucial part of the health care system. Almost 20% of the plants present in the world have been evaluated for pharmacological or biological tests (Suffredini *et al*., 2004). Plant derivatives like honey have shown antibacterial activity against *B. alvei, B. polymyxa, B. Subtilis* and *S. Aureus* (Iqbal *et al.,* 2015). The previous studies of (Hussain *et al.,* 2016) evaluated that the methanol extracts of leaves, stem, roots and seeds of *Datura innoxia* showed activities against tested bacterial species. It was concluded that medicinal plants have huge contribution to the traditional and western medicine by providing ingredients for drug or being used in the drug discoveries. Al-Hakami *et al*., (2016) documented antimicrobial activity of Cinnamon barks (Aqueous and Ethanolic Extracts) against *Candida albicans.*

Sundaram Ravikumar *et al*., (2010) 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. John De Britto and Herin Sheeba Gracelin, (2011) investigated the phytochemicals present in leaves, stem, flowers and fruits of *Datura metel* which have some medicinal applications. The previous studies of Mei X. Chen *et al*., (2016) prepared antibacterial creams and gels containing metal ions for topical application and concluded that copper sulfate and zinc sulfate have a synergistic antibacterial activity in creams and gels.

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