

Analysing the multidrug resistance of microbes against three different ecological adaptations. Hydrophytic adaptation (*Nymphaea nouchali*), Xerophytic adaptation (*Aloe vera*), Mesophytic adaptation (*Tridax procumbens*)

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Abstract

Multidrug resistance is the ability of microorganisms to be resistant to more than two drugs. In this study, the multidrug resistance of microbes against three different ecological adaptations, Hydrophytic adaptations. The study indicated that the plants from xerophytic adaptation shows the higher antimicrobial activity than that of hydrophytic and mesophytic adaptations.

Keywords: xerophytic adaptations, Hydrophytic adaptations, antimicrobial activity, Multidrug resistance

INTRODUCTION

India has a vast and inexhaustible resource of drugs of plant origin and India can supply the drugs to many other countries. Plants have been one of the important sources of medicines even since the dawn of human civilization one of the earliest treatises of Indian medicine, the “charakasamhitha” (100 BC) mention the uses of 2000 vegetable herbs for medicinal use. Over 7000 different ecosystem are said to be used for medicinal purpose in our country [1].

A substantial fraction of the world’s population continues to use material products, especially medicinal plant extract in helping to control infectious diseases and to combat pests. In many cases, however, the knowledge of the correct preparation and uses of these materials is disappearing and the resources themselves are being depleted. In some States of India, pharmaceutical companies are already marketing preparations of tablets, capsules etc. made directly from the appropriate plant extracts for the treatment of specific diseases like Hepatitis [2]. The discovery of medicinal

plants in different part of the world is important both in the agriculture and medicine sectors, in the establishment of new direction towards propagation of alternative medicinal crops that offer better economic and social benefits. Medicinal plants do play an important role in the treatment of ailments of Malaysia. The use of plant preparation for such purposes has been documented [3].

Herbal remedies are "medicines" derived from nature that are used to treat the entire body. Some herbal remedies are taken pure - without changing the physical form of the herb - while others are converted into capsules, drinks, salves and creams. Modern medicine takes chemical and biological compounds and converts the medicinal properties each into liquid and solid medicines, some of which are prescribed, while others can be found over the counter (OTC).

Herbal medicines have been important sources of products for the developing countries in treating common infectious diseases and overcome the problems of resistance and side effects of the currently

available antimicrobial agents. The World Health Organisation (WHO) estimates that 80% of the people living in developing countries almost exclusively use traditional medicines. This means approximately 3300 million people use medicinal plants on a regular basis. Medicinal plants used in traditional medicine should therefore be studied for safety and efficacy [4].

Out of nearly 3,00,000 species of higher plants available, only a small proportion have been investigated for medicinal features, and still smaller number yield well-defined drugs. The same is the case typically with lower plants occur are Fabaceae, Lamiaceae, Euphorbiaceae, Asteraceae, Liliaceae, Poaceae, Rubiaceae, Apiaceae, Cucurbitaceae, Zingiberaceae, Convolvulaceae, Mimosaceae, Malvaceae and Solanaceae [5].

Drugs are derived from trees, shrubs, herbs and even from primitive kinds of plants which do not fall into the above categories. They are mainly made from fruits, flowers, leaves, stems, roots, seeds and even bark.

PLANT MATERIALS USED FOR THE PRESENT STUDY:

Hydrophytic Adaptation

Nymphaea nouchali

Nymphaea nouchali is considered a medicinal plant in Indian Ayurvedic medicine under the name *Ambal*; it was mainly availed to treat indigestion [6]. Recent experiments have confirmed that it has major medicinal qualities as an antihepatotoxic, anthelmintic, antidiabetic. Like all waterlilies or lotuses, its tubers and rhizomes can be availed as food items; they are eaten usually boiled or roasted. In the case of *N. nouchali*, its tender leaves and flower peduncles are also valued as food [7].

Xerophytic Adaptation

Aloe vera

Aloe vera, called *kathalai* in Ayurvedic medicine, is availed as a multipurpose skin treatment. This may be partly due to the presence of saponin, a chemical compound that acts as an antimicrobial agent. It is used on facial tissues where it is promoted as a moisturiser and or anti-irritant to reduce chafing of the nose [8].

Mesophytic Adaptation

Tridax procumbens

Traditionally, *Tridax procumbens* has been in use in India for wound healing, as anticoagulant, antifungal and insect repellent. There is also certain plants which has febrifuge properties [9]. It is also used in diarrhea and dysentery. Its leaf extracts were known to treat infectious skin diseases in folk medicines. It is a well-known ayurvedic medicine for liver disorders or hepato-protective nature besides gastritis and heart burn.

MATERIALS AND METHODS

MATERIALS

The herbal plants from different habitats were selected, extracted and used for further studies. The sample plants were collected from in and outside of the college campus. The plants collected were washed with water and surface sterilized with 0.1 % Mercuric chloride.

THE SAMPLE FROM HYDROPHYTIC ADAPTATIONS:

Sample: *Nymphaea nouchali*

Scientific classification

Class : Dicotyledons

Orde : Nymphaeales

Family: Nymphaeaceae

Genus: *Nymphaea*

Species: *nouchali*

Binomial name: *Nymphaea nouchali* Burn. f.

DESCRIPTION

This aquatic plant is native from the Indian Subcontinent to Australia region. It has been long valued as a beautiful garden flower in Thailand and Myanmar to

decorate ponds and gardens. In its natural state *N. nouchali* is found in static or slow-flowing aquatic habitats of little to moderate depth [10].

Nymphaea nouchali is a day blooming non-viviparous plant with submerged roots and stems. Part of the leaves are submerged, while others rise moderately above the surface. The leaves are round and green on top; they usually have a darker underside. The floating leaves have undulating edges that give them a crenellate appearance. Their size is about 20–24 cm and their spread is 0.9 to 1.9 m [11].

This water lily has a beautiful flower which is usually violet blue in color with reddish edges. Some varieties have white, purple, mauve or fuchsia-colored flowers. The flower has 4-5 sepals and 13-16 petals that have an angular appearance making the flower look star-shaped from above. The cup-like calyx has a diameter of 11–15 cm.

SAMPLE FROM XEROPHYTIC ADAPTATION

SAMPLE : *Aloe vera*

Scientific classification

Class : Monocotyledons

Order: Asparagales

Family : Xanthorrhoeaceae

Sub family : Asphodeloideae

Genus : *Aloe*

Species : *vera*

Binomial name : *Aloe vera* (L) Burm. F.

DESCRIPTION

Aloe vera is a succulent plant species that is seen only in cultivation, having no naturally occurring populations, although closely related aloes do occur in northern Africa [12]. The species is frequently cited as being availed in herbal medicine since the beginning of the first century AD. Extracts from *A. vera* are widely availed in the cosmetics and alternative

medicine industries, being marketed as variously having rejuvenating, healing, or soothing properties. There is, however, little scientific evidence and assessment of the effectiveness or safety of *Aloe vera* extracts for either cosmetic or medicinal purposes, or what positive evidence is available is frequently contradicted by other studies [13].

It is a stemless or very short-stemmed succulent plant growing to 60–100 cm (25–39 in) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on their upper and lower stem surfaces. The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up to 90 cm (36 in) tall, each flower being pendulous, with a yellow tubular corolla 2–3 cm (0.8–1.4 in) long. Like other *Aloe* species, *Aloe vera* forms arbuscular mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil [14].

SAMPLE FROM MESOPHYTIC ADAPTATION

SAMPLE : *Tridax procumbens*

Scientific classification

Class : Dicotyledons

Order : Asterales

Family : Asteraceae

Tribe : Heliantheae

Genus : *Tridax*

Specie : *procumbens*

Binomial name : *Tridax procumbens* L.

DESCRIPTION

Tridax procumbens is a species of flowering plant in the daisy family. It is best known as a vast spread weed and pest plant. It is native to the tropical Americas but it has been introduced to tropical, subtropical, and mild temperate regions worldwide. It is listed as a noxious weed majorly in the United States and has pest status in nine states [15].

The plant bears daisy like yellow-centered white or yellow flowers with three-toothed ray florets. The leaves are toothed and generally arrowhead-shaped. Its fruit is a hard achene covered with stiff hairs and having a feathery, plume like white pappus at one end. Calyx is majorly represented by scales or reduced to pappus. The plant is invasive in part because it produces so many of these achenes, up to 1500 per plant, and each achene can catch the wind in its pappus and be carried some distance. This weed can be found in fields, meadows, croplands, disturbed areas, lawns, and roadsides in areas with tropical or semi-tropical climate [16].

MICRO ORGANISM USED FOR THE PRESENT STUDY

Gram positive bacteria – *Staphylococcus aureus*

Staphylococcus aureus in Greek “grape – cluster berry” is a facultative anaerobic gram-positive cocci bacterium also known as “golden staph”. It was initially discovered in 1880 in Aberdeen, United Kingdom, by the surgeon Sir. Alexander Ogston in pus from surgical abscess in a knee joint. It is a bacterium which is frequently seen in human respiratory tract and on the skin. Although it is not always pathogenic, it is common cause of skin infections, respiratory diseases and food poisoning [17].

MATERIALS REQUIRED

Conical flask (250 ml), petri plates, beaker, test tubes, watch glass, sterilized Watman filter paper disc, nutrient agar medium, 24 hour old bacterial culture of *Staphylococcus aureus*, swab, alcohol for sterilization, spirit lamp, forceps, incubators.

METHODOLOGY

PREPARATION OF EXTRACTS

The leaves from all the sample plant were collected separately, washed, surface sterilized and made into extracts with different solvents like Methanol and Aqueous.

BACTERIAL CULTURE

The bacteria *Staphylococcus aureus*, was isolated from Industrial effluent through pour plate method.

PREPARATION OF BROTH

Take 4.5ml of distilled water, to this add pinch of nutrient agar and mix well. From the bacterial culture is transferred to the culture tubes with help of an inoculation loop. Plug the test tube tightly. Under sterile conditions, with help of inoculating loop transfer the bacteria from the pure culture to the plate by quadrant streaking and incubate it for 24 hours at 37^o C.

DISC DIFFUSION METHOD

Disc diffusion method for antimicrobial susceptibility testing was carried out according to standard methods, to assess the multidrug resistant activities of the selected bacteria against plant extracts. Watman No.1 filter paper is used as filter paper disc. They were placed on the medium containing bacteria. Then the discs were soaked into the plant extracts in a watch glass and without plant extracts were also maintained [18].

PREPARATION OF CULTURE PLATES

Required amount of nutrient agar is weighed and added to 100 ml distilled water and is sterilized using autoclave. After sterilization pour this media into the petri plates under sterile conditions. Allow it to solidify. Prior to sensitivity testing, the selected pure culture of bacteria from previously prepared broth was spread uniformly over the prepared nutrient agar plates. For this, moisten a sterile swab by immersing it into a tube of nutrient broth containing *S. aureus* culture. Spread the culture broth on the medium with the help

of swab. The Watman filter paper disc was placed aseptically on the sterile forceps. Then the disc was impregnated with the series of plant extracts [19].

INCUBATION

Plates were then incubated at 37^o C for about 24 hours in upright position. After incubation, the plates were examined for inhibition zone. In order to ensure reliability, the test were repeated.

MICROSCOPIC AND BIOCHEMICAL EXAMINATION

In order to identify the major pathogens the microscopic examination such as gram staining and biochemical characterization such as IMViC, Voges Prausker, Citrate utilization test, Carbohydrate fermentation test, Catalase , Oxidase test are carried out [20].

RESULT AND DISCUSSION

The efficiency of multidrug resistant *Staphylococcus aureus* were tested against the three different sample plant collected from different environmental adaptations.

Isolation and cultivation of Bacteria

The multidrug resistant microbe was isolated from Industrial effluent through Pour plate method (Plate- 2). The isolated organism were subjected for various preliminary examinations to confirm its presence. After confirming, the microbes were stored in selective media for further use (Plate-3).

Confirmatory Test

Staphylococcus aureus was subjected for Coagulase Test for Slide and Tube test were coined out.

Slide Test: Rapid clumping of the suspension occurs with Coagulase.

Tube Test: Clotting of plasma occurred in continued incubation for more than 6 hours.

Isolation of MRSA by Disc Diffusion Test:-

The isolated microbes are confirmed by Oxacillin Screening Test.

Double Disc Diffusion Synergy Test (DDST):-

The *Staphylococcus aureus* was subjected to synergy test, to get confirm with its multidrug resistance capacity. This test was done normally with clinically isolated strains. But this was applied for the industrial effluent isolated strains, slide showed positive results

Extended Spectrum β - Lactamases (ESBL) are Ambler class A penicillin uses which confer resistance and hydrolyze the expanded – spectrum cephalosporin like ceftazidime, cefotaxime, meropenem, aztreonam amino and related oxymino β -lactams as well as older penicillin and cephalosporins.

Antimicrobial activity

The antimicrobial activity of three plants belonging to different ecological adaptations was evaluated in vitro against multidrug resistant *Staphylococcus aureus*. The antimicrobial activity of the extracts and their potency was assessed by the presence or absence of inhibition zone as given fig: 1-3. The greater inhibition was shown by aqueous extracts of *Aloe vera*, the least inhibition was shown by *Tridax procumbens* and both aqueous and methanolic extracts of *Nymphaea nouchali* shows zone of inhibition in between these two plants. The methanolic extracts of *Aloe vera* shows higher rate of inhibition that of *Nymphaea* and *Tridax*.

Biplab Kumar et al. in 2013, were studied about the antimicrobial activity of *Nymphaea nouchali* flower. In that study they observed that the methanol extracts possessed better antibacterial activity against two pathogenic bacteria. The antimicrobial activity of *Tridax procumbens* by [11]. They confirmed that the aqueous extracts of *T. procumbens* show good antimicrobial activity.

Antimicrobial activity of *T.procumbens* with nosocomial pathogen by Chitra Pai et al., 2011 [12], confirmed that aqueous extract had no antimicrobial activity but the

alcoholic extract significantly effective against antibiotics and they concluded that *Tridax* leaves may useful multidrug resistant against pathogen.

PLANTS FROM DIFFERENT ADAPTATIONS



Nymphaea nouchali – Hydrophyte



Xerophyte- *Aloe vera*



Tridax procumbens - Mesophyte

ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS



Fig: 1. *Nymphaea* extract



Fig: 2. *Aloe vera* extract



Fig: 3. *Tridax* extract

CONCLUSION

All these plants extract tested have traditional claims for antimicrobial activity and these findings were in with their therapeutic properties for antibacterial as claims. From the present study the plants from different ecological adaptation showed greater activity against multidrug resistant *Staphylococcus aureus*. MRSA showed great zone of extend of inhibition aqueous extract of *Aloe vera*. *Nymphaea nochali* and *Tridax procumbens* showed minimum activity both in aqueous and methanol extracts. Therefore it was concluded that the plants from xerophytic adaptation shows the higher antimicrobial activity than that of hydrophytic and mesophytic adaptations.

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