

## Antibacterial activity of leaves of *Nyctanthus arbor-tristis* L, *Hibiscus rosa-sinensis* L. and *Sapindus emarginatus* Vahl.

M. B. Patil<sup>1</sup> and Pathan Amanulla Khan<sup>2</sup>



### ABSTRACT

Three plants were screened for potential antibacterial activity. In evaluating antibacterial activity both aqueous and organic solvents were used. The plants screened were *Hibiscus rosa-sinensis*, *Nyctanthus arbor-tristis* and *Sapindus emarginatus*. Antibacterial activity was tested against two bacterial strains, *Staphylococcus aureus* and *S. epidermidis*. Two methods, Agar disk diffusion and Agar ditch diffusion, were used to study the antibacterial activity of three plants. *S. emarginatus* showed strong activity against the tested bacterial strains. Therefore this can be selected for further investigation to determine its therapeutic potential.

#### Keywords:

*Hibiscus rosa-sinensis*, *Nyctanthus arbor-tristis*, *Sapindus emarginatus*, and antibacterial activity.

#### Introduction

With the great advancement in science in also in medicine, the word is emerging in the grip of dreadful fear of development of resistance in antibiotics by the microorganisms. For the treatment of infectious diseases, finding out the substitute from the nature to the antibiotics is becoming the prime need of the society in the present and future. *Hibiscus rosa-sinensis*, *Nyctanthus arbor-tristis* and *Sapindus emarginatus* are used to cure number of skin infections. Charadatta mentions the treatment of fever, rheumatism and nematodes infestation.

Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry. There has been a revival of interest in herbal medicines. This is due to increase in awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom. Plants are the basic source of knowledge of modern medicine. The basic molecular and active structure for synthetic fields is provided by rich natural sources. This world wide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care. The relatively lower incidence of adverse reactions to plant preparation compared to modern conventional pharmaceuticals, coupled with their reduced cost. Which are encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs. Plants with possible antimicrobial activity should be tested against an appropriate microbial associated with it. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Much work has been done on ethno medicinal plant in India. Interest in a large number of traditional natural products has increased. It has been number of traditional natural products has increased. It has been also suggested that aqueous and ethanolic extracts from plants used in allopathic medicines are potential sources of antiviral, antitumoral and antimicrobial agents. In the

M. B. Patil<sup>1</sup> and Pathan Amanulla Khan<sup>2</sup>

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Department of Botany  
Jijamata Education Society's,  
Arts Science and Commerce College,  
Nandurbar, Maharashtra.

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present work a few selected medicinal flora were screened for potential antibacterial activity.

### Screening of medicinal plants

#### *Nyctanthes arbortristis* L. (MBP-58)

*Nyctanthes arbortristis* Linn. Belonging to the family Oleaceae, locally known as Parijatak. The tree measures up to 3-10 m in height. The leaves face forwards and are 10-12.5 cm long. The leaf juice is used to treat loss of appetite, piles, liver disorders, biliary disorders, intestinal worms, chronic fever, obstinate sciatica, rheumatism and in alopecia. It is antibilious and an expectorant and also useful in bilious fevers.

#### *Hibiscus rosa-sinensis* L. (MBP-41)

*Hibiscus rosa-sinensis* L. belongs to the family Malvaceae locally known as Jaswand. The roots are cylindrical, 5-15 cm in length and 2 cm in diameter, off white and with light brown transverse lenticels. The roots taste and are mucilaginous, leaves are simple ovate or ovate-lanceolate, and are entire at the base and coarsely toothed at the apex. The flowers are pedicellate, actinomorphic, pentamerous and complete. The corolla consists of 5 petals, red and about 8 cm in diameter. Traditionally this plant is used for the control of dysfunctional uterine bleeding and as an oral contraceptive. Some of the chemical constituents isolated from this plant are cyaniding, quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin and ascorbic acid. Flavonoids are also present.



#### *Sapindus emarginatus* Vahl. (MBP-25)

*Sapindus emarginatus* Vahl. Belongs to the family Sapindaceae and locally known as Ritha. This tree is 8-10 m in height with many branches with leaves and leaflets. Its flowers are white and fruits are round. It contains saponin and glucose. The seeds contain oil. Traditionally, it is used as anti-inflammatory and antipruritic. It is used to purify the blood. The seed is intoxicant and the fruit rind has oxytropic action. Its powder is used as nasal insufflations.

### MATERIAL AND METHODS

Fresh plant or parts were collected randomly from fields near by the Nandurbar city. The taxonomic identification of these plants was done by Department of Botany, Jijamata Education Society's, Arts, Science and Commerce College, Nandurbar-425412, Maharashtra. Fresh material were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

**Aqueous extraction:** For aqueous extraction, 10 g of air-dried powder was placed in distilled water and boiled for 6 h. At intervals of 2h it was filtered through 8 layers of muslin cloth and centrifuged at 5000 x g for 15 min. the supernatant was concentrated to make the

final volume one-fourth of the original volume. Finally 10 g of material was extracted in 25 ml of distilled water giving a concentration of 40 mg /0.1 ml. It was then autoclaved at 121C and 15 lbs pressure and stored at 4C.

**Solvent extraction:** Ten grams of air dried powder was placed in 100 ml of organic solvent (methanol) in a conical flask, plugged with cotton and then kept on a rotary shaker at 190-220 rpm for 34 h. After 24 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 x g for 15 min. The supernatant was collected and solvent was evaporated to make the final volume one-fourth of the original volume, giving a concentration of 40 mg/0.1 ml. It was stored at 4 C in airtight bottles for further studies.

**Test microorganisms:** The microbial strains are identified strains and were obtained from the National Chemical Laboratory (NCL), Pune, India. The bacterial strains studied are *Staphylococcus aureus* and *S. epidermidis*

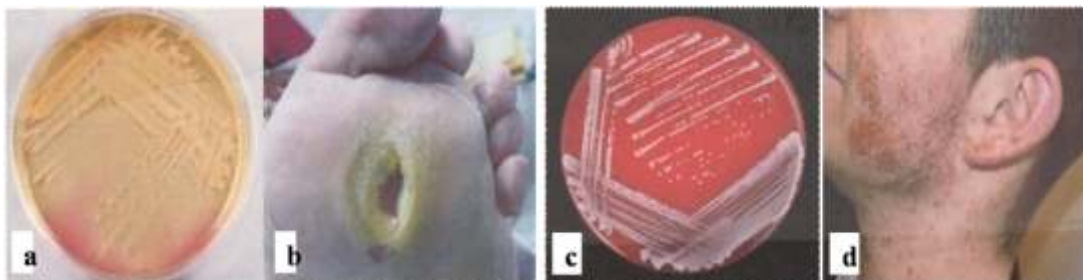


Fig: (a) *Staphylococcus aureus*, (b) Wound due to infection of *Staphylococcus aureus*, (c) *Staphylococcus epidermidis* (d) Skin disease due to infection of *Staphylococcus epidermidis*

**Antibacterial assay:** A loop full of the strain was inoculated in 30 ml of nutrient broth in a conical flask and incubated on a rotary shaker for 34 h to activate the strain. Mueller Hinton Agar No.2 was prepared for the study. The assay was performed using 2 methods. Ager disk diffusion for aqueous extract and Agar ditch diffusion for solvent extract. The media and the test bacterial cultures were poured into Petri dishes (HI-Media). The test strain (0.2 ml) was inoculated into the media (inoculum size 10<sup>8</sup> cells/ml) when the temperature reached 40-42 C. Care was taken to ensure proper homogenization. The experiment was performed under strict aseptic condition. For the Ager disk diffusion method, the test compound (0.1 ml) was introduced onto the disk (0.7 cm) (Hi-media) and then allowed to dry. Thus the disk was completely saturated with the test compound. Then the disk was introduced onto the upper layer of the medium with the bacteria. The plates were incubated overnight at 37 C. For the Agar ditch diffusion method, after the medium was solidified, a ditch was made in the plates with the help of a cup-borer (0.85 cm). The test compound was introduced into the well and the plates were incubated overnight at 37 C. Microbial growth was determined by measuring the diameter of the zone of inhibition. Methanol and distilled water were used as the control. The control activity was deducted from the test and the result obtained was plotted.

## RESULTS AND CONCLUSIONS

The antibacterial activity of *S. emarginatus* leaf extract of both solvents (aqueous and methanolic) against *staphylococcus aureus* and *S. epidermidis*. The methanolic extract showed considerably more activity than the aqueous extract. Maximum antibacterial activity was shown against *Staphylococcus epidermidis*. Neither aqueous nor methanolic extracts were able to inhibit both of the tested bacterial strains. The aqueous extract showed some activity against *S. aureus* but showed negligible activity against *S. epidermidis*. This plant, i.e. *N. arbortristis*, extract (methanol), was unable to inhibit both of the bacterial strains studied.

The aqueous extract appears to have less antibacterial activity than the methanolic extract. This is interesting in that the traditional method of treating a bacterial infection

was by administering a decoction of the plant or a part there of by boiling it in water , whereas according to our results an organic solvent is better; hence this may be more beneficial. *S. emarginatus* showed maximum antibacterial activity and so this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither to unmet therapeutic needs.

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M. B. Patil

Department of Botany , Jijamata Education Society's, Arts Science and Commerce College,  
Nandurbar, Maharashtra.



Pathan Amanulla Khan

Department of Botany , Jijamata Education Society's, Arts Science and Commerce College,  
Nandurbar, Maharashtra.