

Antibacterial Activity of Some Selected Indian Medicinal Flora

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Abstract: Nine plants were screened for potential antibacterial activity. In evaluating antibacterial activity both aqueous and organic solvents were used. The plants screened were *Sapindus emarginatus*, *Hibiscus rosa-sinensis*, *Mirabilis jalapa*, *Rheo discolor*, *Nyctanthes arbortristis*, *Colocasia esculenta*, *Gracilaria corticata*, *Dictyota* spp., and *Pulicaria wightiana*. Antibacterial activity was tested against 6 bacterial strains, *Pseudomonas testosteroni*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Proteus morgani*, and *Micrococcus flavus*. Two methods, Agar disk diffusion and Agar ditch diffusion, were used to study the antibacterial activity of all these plants. *Ps. testosteroni* and *K. pneumoniae* were the most resistant bacterial strains. *S. emarginatus* showed strong activity against the tested bacterial strains. Therefore, this can be selected for further investigation to determine its therapeutic potential. Its leaf extract can also be used as a lead molecule in combating the diseases caused by the bacterial strains studied.

Key Words: *Sapindus emarginatus*, *Hibiscus rosa-sinensis*, *Mirabilis jalapa*, *Rheo discolor*, *Nyctanthes arbortristis*, *Colocasia esculenta*, *Gracilaria corticata*, *Dictyota* spp., *Pulicaria wightiana*, antibacterial activity.

Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible, has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (1). Over 50% of all modern clinical drugs are of natural product origin (2) and natural products play an important role in drug development programs in the pharmaceutical industry (3).

There has been a revival of interest in herbal medicines. This is due to increased 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 structures for synthetic fields are provided by rich natural sources. This burgeoning worldwide interest in medicinal plants reflects a 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 preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is 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 model to confirm the activity and to ascertain the parameters 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 (4-6). Much work has been done on ethnomedicinal plants in India (7-9). Interest in a large number of traditional natural products has increased (10). It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents (11,12). The selection of crude plant

extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products (13).

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

Screening of medicinal plants

Sapindus emarginatus

Sapindus emarginatus Vahl. (PSN-131) belongs to the family Sapindaceae. This tree is 8 to 10 m high and has many branches with leaves and leaflets. Its flowers are white, and its fruits are round. It contains saponin and glucose. The seed contains oil. Traditionally, it is used as anti-inflammatory and antipruritic. It is used to purify the blood. The seed is in intoxicant and the fruit rind has oxytropic action. Its powder is used as a nasal insufflation.

Hibiscus rosa-sinensis

Hibiscus rosa-sinensis (PSN-57) belongs to the family Malvaceae. The roots are cylindrical, 5-15 cm in length and 2 cm in diameter, off white and with light brown transverse lenticles. The roots taste sweet and are mucilaginous. The 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 cyanidin, quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin and ascorbic acid. Flavonoids are also present.

Mirabilis jalapa

Mirabilis jalapa Linn. (PSN-634) belongs to the family Nyctaginaceae. It is a large herbaceous plant grown in gardens throughout India. This plant is 50-100 cm high. It has antifungal, antimicrobial, antiviral, antispasmodic, antibacterial, diuretic, carminative, cathartic, hydragogues, purgative, stomachic, tonic and vermifuge properties (14) This plant contains alanine, alpha-amyrins, arabinose, beta-amyrins, campesterol, daucosterol and dopamine (15), and is used to treat conjunctivitis, edema, fungal infections, inflammation, pains and swellings.

Rheo discolor Hance

Rheo discolor Hance belongs to the family Commelinaceae. It is commonly grown in gardens, and is usually known as Tradescantia. The leaves are large, imbricated, green above and purple beneath.

Nyctanthes arbortristis

Nyctanthes arbortristis Linn. (PSN-434) belongs to the family Oleaceae. 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 fever with rigors. The seeds are used as anthelmintics and in alopecia. It is antibilious and an expectorant, and is also useful in bilious fevers.

Colocasia esculenta

Colocasia esculenta Schott. (PSN-748) belongs to the family Araceae. The plant is a hearty succulent herb, with clusters of long heart or arrowhead-shaped leaves that point earthwards. It grows on erect stems that may be green, red black or variegated. The stems are a few meters high. The species is thought to be a native of India. The young leaves are rich in Vitamin C, and the roots are rich in starch. It contains thiamine, riboflavin, niacin, oxalic acid, calcium oxalate and saptotoxin. The tubers contain aminoacids and proteins. The corms contain the anthocyanins perlargonidin 3-glucoside, cyaniding 3-rhamnoside and cyaniding 3-glucoside. Traditionally it is used to settle the stomach, to prevent swelling and pain and to reduce fever. It is also used as a poultice on infected sores.

Gracilaria corticata

Gracilaria corticata belongs to the family Rhodophyceae. These algae are multicellular, forming well-developed branched thalli.

Dictyota spp.

Dictyota spp. belong to the family Phaeophyceae. The brown algae vary in size from microscopic plants with a few cells to very large plants some 70 m in length. Most brown algae possess a holdfast, stipe and blade.

Pulicaria wightiana

Pulicaria wightiana (PSN-403) belongs to the family Asteraceae. The leaves are sessile, and pubescent on both sides.

Table 1. Screening of some medicinal plants for potential antibacterial activity.

No.	Botanical name	Family	Vernacular name	Parts used
1.	<i>Sapindus emarginatus</i>	Sapindaceae	Aritha	Leaf
2.	<i>Hibiscus rosa-sinensis</i>	Malvaceae	Jasud	Leaf
3.	<i>Mirabilis jalapa</i>	Nyctaginaceae	Gulbas	in toto
4.	<i>Rheo discolor</i>	Commelinaceae	-	Leaf
5.	<i>Nyctanthes arbortristis</i>	Oleaceae	Parijatak	Leaf
6.	<i>Colocasia esculenta</i>	Araceae	-	Leaf
7.	<i>Gracilaria corticata</i>	Rhodophyceae	-	in toto
8.	<i>Dictyota</i> spp.	Phaeophyceae	-	in toto
9.	<i>Pulicaria wightiana</i>	Asteraceae	Shinshoria	in toto

A number of plants were therefore screened for potential antibacterial activity.

Materials and Methods

Fresh plants or parts there of were collected randomly from the semi-arid region of Rajkot Gujarat, India, in August, 2003. The taxonomic identities of this plant were determined by Dr. P.S. Nagar, the taxonomist at the Department of Biosciences, Saurashtra University, Rajkot. Fresh plant materials 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 2 h it was filtered through 8 layers of muslin cloth and centrifuged at 5000 x g for 15 min. The supernatant was collected. After 6 h, 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 121 °C and 15 lbs pressure and stored at 4 °C.

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 24 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 the 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 *Pseudomonas testasteroni* NCIM 5098, *Staphylococcus epidermidis* ATCC 12228, *Proteus morgani* NCIM 2040, *Bacillus subtilis* ATCC 6633, *Micrococcus flavus* ATCC 10240 and *Klebsiella pneumoniae* NCIM 2719.

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 24 h to activate the strain. Mueller Hinton Agar No. 2 was prepared for the study. The assay was performed using 2 methods. Agar disk diffusion (16) for aqueous extract and Agar ditch diffusion (17) 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 10^8 cells/ml) when the temperature reached 40-42 °C. Care was taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions.

For the Agar 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 *Ps. testosteroni*, *K. pneumoniae*, *M. flavus*, *P. morgani*, *B. subtilis* and *S. epidermidis* is shown in Figure 1a. The methanolic extract showed considerably more activity than the aqueous extract. Maximum antibacterial activity was shown against *M. flavus*, followed by *S. epidermidis* and *P. morgani*. Neither of the extracts were able to inhibit *Ps. testosteroni* or *K. pneumoniae*. The antibacterial activities of *H. rosa-sinensis* and *M. jalapa* are shown in Figure 1b and Figure 1c, respectively. Neither aqueous non methanolic extracts were able to inhibit any of the tested bacterial strains.

In Figure 2a the antibacterial activity of *R. discolor* against the tested bacterial strains is shown. Neither of

the extracts (aqueous or methanolic) were able to inhibit any of the tested bacterial strains. The antibacterial activity of *N. arbortristis* against the tested strains is shown in Figure 2b. The aqueous extract showed some activity against *Ps. testosteroni*, but showed negligible activity against the other bacterial strains. This plant, i.e. *N. arbortristis*, extract (methanolic), was unable to inhibit any of the bacterial strains studied. The antibacterial activity of *C. esculenta* is shown in Figure 2c. The aqueous extract did not show any activity against any of the bacterial strains. The methanolic extract showed inhibitory activity against *K. pneumoniae* only, and none of the other bacterial strains were affected.

The antibacterial activity of *G. corticata* is shown in Figure 3a. Neither of the extracts were able to inhibit any of the tested bacterial strains. In Figure 3b, the antibacterial activity of *Dictyota* spp. is shown. The aqueous extract showed slight activity against *S. epidermidis* and *B. subtilis*, whereas methanolic extract showed activity against *B. subtilis* only. Both the extracts

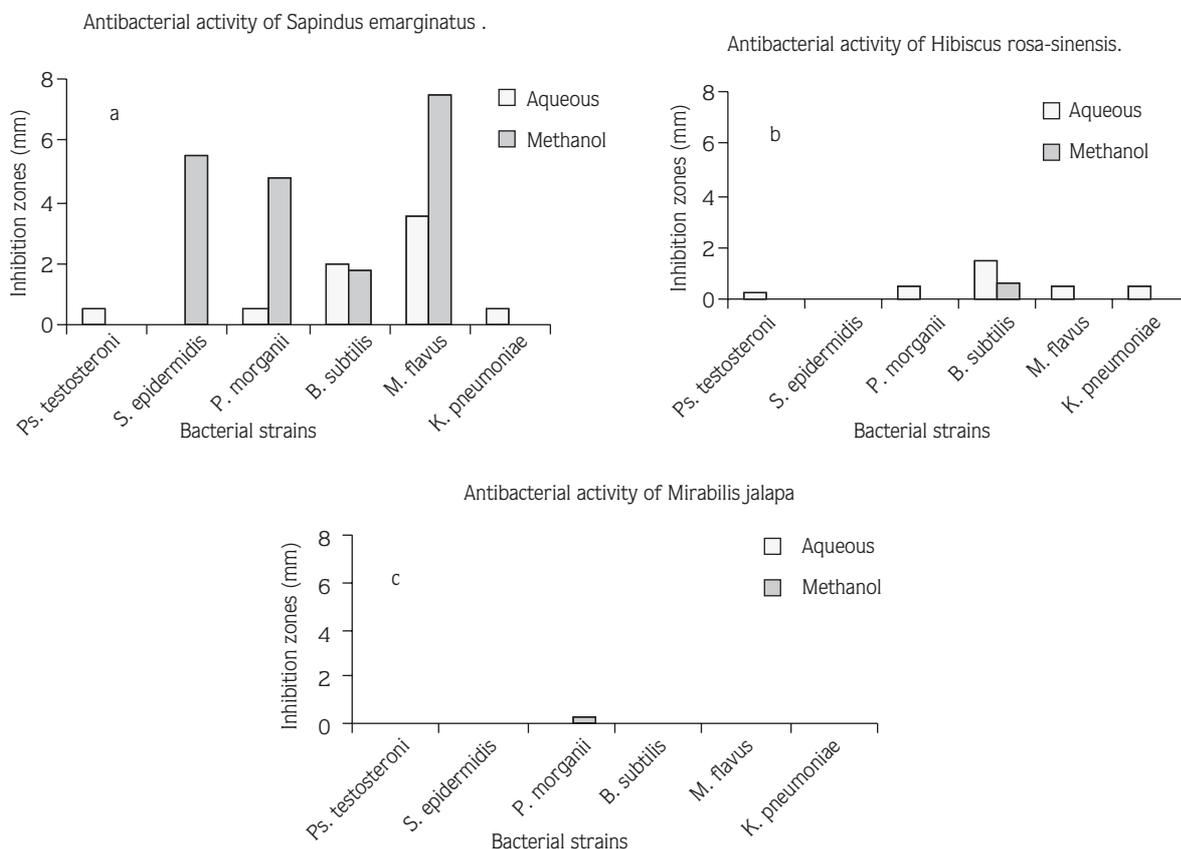


Figure 1. Antibacterial activity of Sapindus emarginatus (a) Hibiscus rosa-sinensis (b) and Mirabilis jalapa (c) in aqueous and methanol extracts against some bacterial strains.

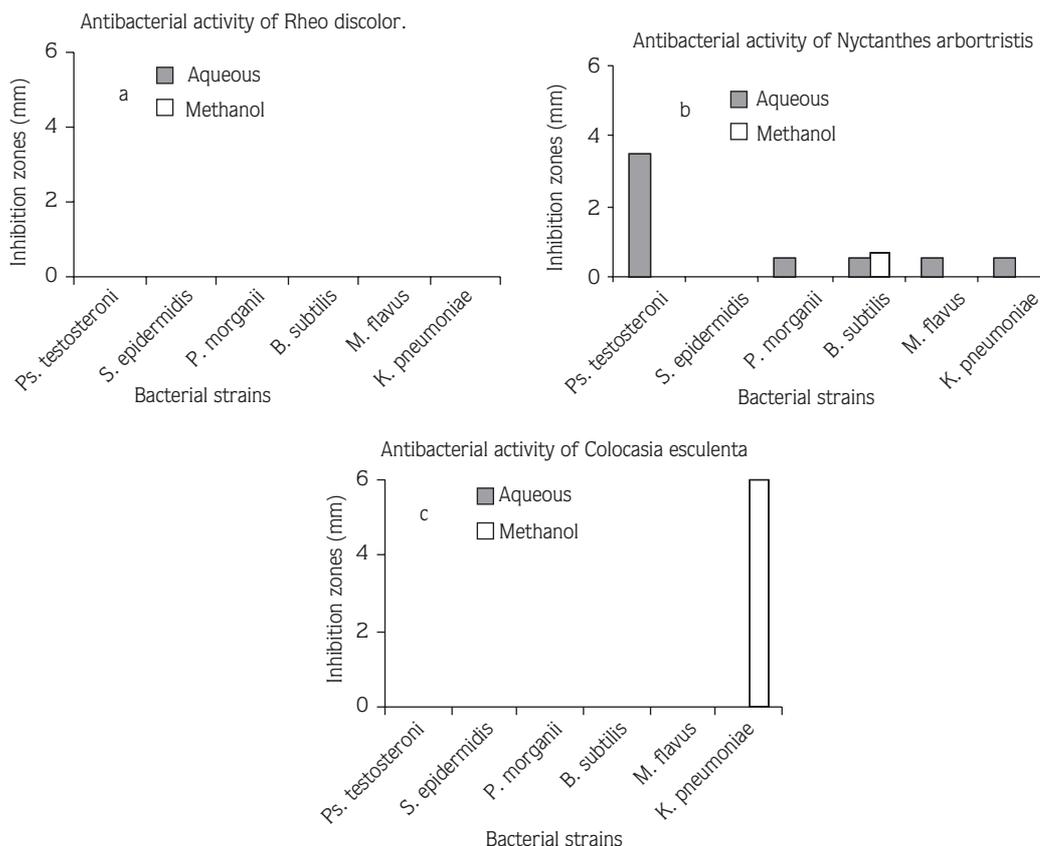


Figure 2. Antibacterial activity of Rheo discolor (a) Nyctantes arbortristis (b) and Colocasia esculenta (c) in aqueous and methanol extracts against some bacterial strains.

showed activity against *B. subtilis*. Neither of the extracts were able to inhibit *Ps. testosteroni*, *P. morganii*, *M. flavus* or *K. pneumoniae*. In Figure 3c the antibacterial activity of *P. wightiana* is shown. The methanolic extract showed more activity than the aqueous extract. The greatest activity of the methanolic extract was against *B. subtilis*, followed by *M. flavus* and *P. morganii*. The other 2 bacteria were not affected. The aqueous extract showed negligible activity.

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. Amongst the 6 bacterial strains investigated *Ps. testosteroni* and *K. pneumoniae* were the most resistant. It was also evident that Gram negative bacteria were more resistant than Gram positive bacteria.

From the above results it can be concluded that plant extracts have great potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by resistant microorganisms. *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. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

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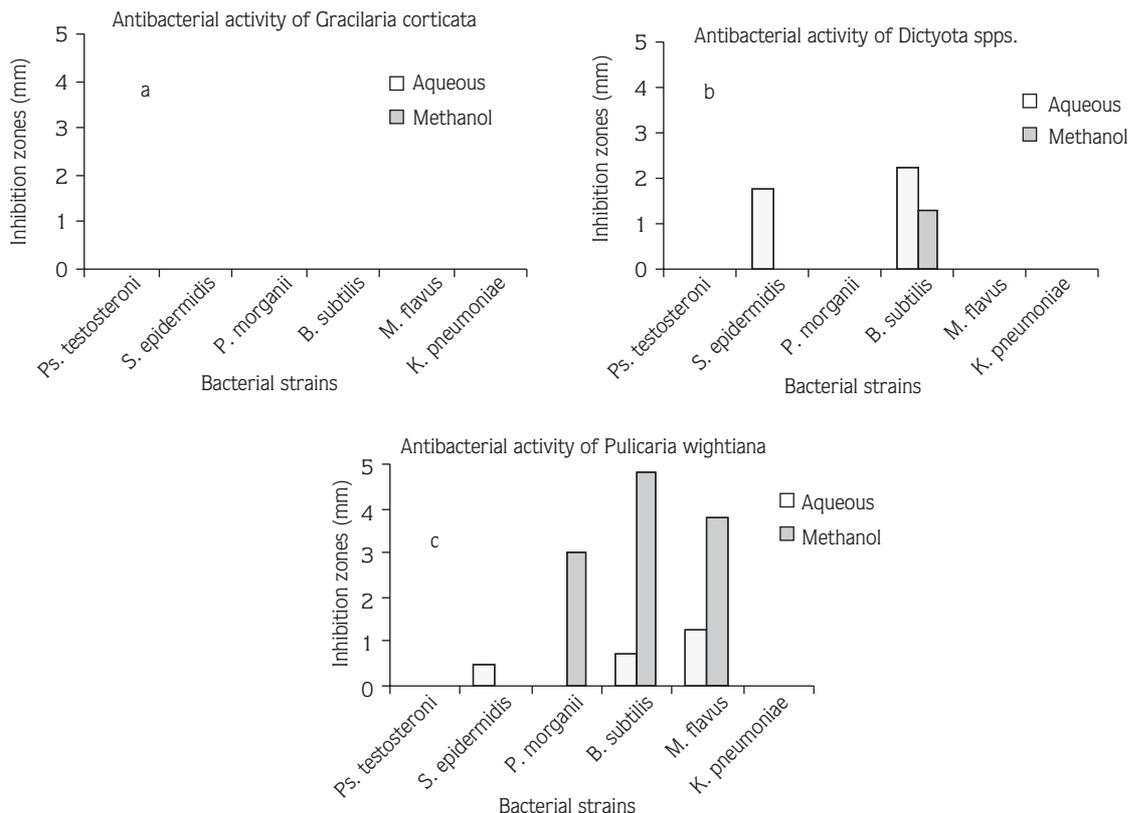


Figure 3. Antibacterial activity of Gracilaria corticata (a) Dictyota spp. (b) and Pulicaria wightiana (c) in aqueous and methanol extracts against some bacterial strains.

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