



SENSITIVITY PATTERNS OF SOME FLOWERING PLANTS AGAINST SALMONELLA TYPHI AND PSEUDOMONAS AERUGINOSA

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ABSTRACT

The medicinal plants have been collected from wild resources and only some species used in larger quantities are cultivated systematically. Many medicinal plants, which were ignored in the past years, but the number of plants still formed in the wild is progressively declining the medicinal value with this situation to acquiring from the wild sources in more important recent trends being. Reported in creasingly the antimicrobial properties of medicinal plants from different parts of the world. On this basis, the present investigation was focused on the screening of antimicrobial properties of *Asystasia indica*, *Asystasia gangetica*, *Thunbergia alata* on selected Pathogenic bacteria. The main objectives of this work use as follow To Screen the antimicrobial properties of these plants on selected micro organisms. To determine the effectiveness of inhibition on microbes by comparing its activity with known antibiotics.



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INTRODUCTION

'Life is gift of God'. This proverb proves that life is very precious and valuable than anything else. Although infectious diseases are the world's leading cause of premature deaths, killing almost 50000 people every day. In recent years, drug resistant to human pathogenic bacteria has been commonly reported from all over the world Radhika et al 2002.,shind et al 2003.,semic et al 2002.,Rals et al 2002.,theodor et al 1991., Lopez-Garcia, et al 1992Plants have been widely used as medicine directly or utilized as a source of medicinal compounds. Traditionally used medicinal plants produce a variety of compounds of know Therapeutic properties (lyengar, 1985; Chopra *et al.*, 1992; Harborne and Baxter, 1995). The substances that can either inhibit the grown of pathogens or kill them and have no or least toxicity to host cells are considered criteria for developing new antimicrobial drugs.

India is blessed with diversity of plant species, which have good medicinal value. Ayurveda, is of plant origin, the Indian indigenous system of medicine, dating back to the Vedic ages (1500-800 BC) has been an integral part of Indian Culture . In Ayurveda, either the whole Plant or Certain parts of leaf, flower, fruits, seeds, heartwood, stem bark, root, gum and resin, have been used for treatment of diseases. The plants over 7000 different species found in the different ecosystems are said to be used for medicinal purposes in India All India Ethnobiological Research project under the ministry of Environment and Forests, Government of India has recently estimated that nearly 8000 medicinal plants are put in used in one way or other in Preparation of medicine and medicinal Compounds in India itself.

Plants containing valuable secondary metabolites and a very wide variety of chemicals that are not directly concerned with primary metabolic Process. They extracted from plants to be used as medicinal agents, Vitamins, insecticides and fine chemicals. Those of particular chemicals are often characteristic of certain genera or families of plants.Medicinal plants produce secondary metabolites namely terpenes and Terpenoids against different pathogenic bacteria .Over the past 10 years there has been a considerable interest in the use of herbal medicines all over the world. Plants may offer new potential sources of active compounds against bacterial, fungal and viral diseases all over the world. Plants derived medicines have been a part of traditional health care in most part of the world for thousands of years and there is an increasing interest in plants as sources of agents to fight against microbial diseases (charinandy et al,1999).

This use of medicinal plants in antimicrobial activites may be attributed to the presence of the antimicrobial compounds like terpenes, essential oils, phenols, certain alkaloids and resins .Recognition of traditional systems of medicines because of their time tested efficacy without side effects and availability at low costs resulted in a great demand for medicinal plants and their products. According to semic et al 2002. theoretically there is a possibility of discovering 328 new modern drugs which are lying hidden in 3,25,000 species of the tropical rain forest, besides the 47 major modern pharmaceutical plant based drugs which are already on the world market medicinal plants therefore, form and important part of international commerce.India can capitalize on her natural inherent strengths and corner a large part of the growing trade provided we remain price competitive and ensure quality

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MATERIALS AND METHODS

Plant material

The following plants were collected from their natural habitats and used for their activity.*Asystasia indica* *Asystasia gangetica* *Thunbergia alata* at the College campus, Kodaikanal in Tamil Nadu.

The plants were shade dried at ambient Temperature (31° C) and the dried materials were crushed into fine powder using an electric blender.

Extract preparation

100 g of dried powder materials of Leaf, Stem, Root, Tuber were soaked separately in 500 ml of each solvent viz. ethanol, chloroform and petroleum ether in conical flasks. Each mixture was stirred at 24 hr using sterile glass rod. It was kept for 72 hrs. At the end of 72 hrs each of the extract was passed through the Whatman No.1 filter paper and the filtrates were concentrated by opening the cover to 1 hr at room temperature in order to reduce the volume. The paste like extracts were stored in labeled screwed chapped bottles and kept in refrigerator at 4° C. Each of the extract was individually reconstituted using minimal amounts of the tracking solvent prior to use.

Microorganisms selected for study

The microorganisms used in this study were collected from Eumic lab, Tiruchirappalli. The selected microbial strains were,

Salmonella typhi,*Pseudomonas auroginosa*



The bacterial strains were maintained in Nutrient agar plate.

Preparation of the media

a) Nutrient agar medium

Composition	-	(1 Litre)
Peptone	-	5.0 gms
Beef extract	-	3.0 gms
Yeast extract	-	1.5 gms
Sodium Chloride	-	5.0 gms
Agar	-	15 gms
Distilled water	-	1000 ml.

After adding all the ingredients into the distilled water boiled to dissolve the medium completely and sterilized by autoclave at 121°C, 15 lbs pressure for 15 minutes.

b) Nutrient Broth (N.B) PH 7.0

Peptone	-	5.0 gms
Beef extract	-	3.0 gms
Sodium Chloride	-	5.0 gms
Yeast extract	-	1.5 gms
Distilled water	-	1000ml.

After adding all the ingredients into the distilled water boiled to dissolve the medium completely and sterilized by autoclave at 121°C, 15 lbs pressure for 15 minutes.

The following methods were selected for antimicrobial studies:

1. Disc diffusion method
2. Streak plate method

Disc Diffusion Method

Principle

The disc-diffusion method provides a simple and reliable test in routine clinical bacteriology in order to find out the effect of a particular substances on a specific bacterium.

This method consists of impregnating small circular discs of standard filter paper with given amount of a chosen concentration of substances. The discs are placed on plates of culture medium. Previously spread with a bacterial inoculum to be tested. After incubation the degree of sensitivity is determined substances from the discs into the surrounding medium.

Preparation of Discs

Discs usually consists of absorbent paper impregnated with the compound (Plant extract). It is most convenient to use Whatman No.1 filter paper for preparing the discs. Dry discs of 6 mm diameter were prepared from Whatman No.1 filter paper and Sterilized in an auto clave. These dry discs were used for the assay.

Procedure

Circular discs of 6 mm diameter were prepared from Whatman No.1 filter paper and Sterilized in an autoclave. These paper discs were impregnated with test compounds (Plant extracts) in the respective solvents for over night and placed on nutrient agar plates seeded with the test bacterium. Preparation of the test solution of different concentration for streak plate method.

Each extract is taken for activity studies, the extracts concentration is made into solution of different concentration as give bellow.

- [25%] 0.5 ml of extract + 1.5 ml of distilled water
- [50%] 1 ml of extract + 1ml of distilled water
- [75%] 1.5 ml of extract +0.5 ml of distilled water
- [100%] 2 ml of pure extract only.



Preparation of control

The control was prepared with Nutrient Agar medium without plant extract. Each of above concentration was tested for antibacterial activity using the *Asystasia indica* *Asystasia gangetica* *Thunbergia alata* against test bacteria *Salmonella typhi* *Pseudomonas aeruginosa*

The plates were incubated at 37° C for 24 hr, after 24 hr the zone of inhibition around each disc was measured and the diameter was recorded. The tests were repeated 4 times to ensure reliability of the result.

Preparation for control

The control was prepared with Nutrient Agar medium without plant extract. Tested for antibacterial activity using standard antibiotic disc. i.e, *ciprofloxacin*.

Streak plate method

The streak plate assay was generally used to obtain discrete colonies and pure colonies. This assay was used to determine the growth inhibition of bacteria by plant extracts. A sterilized loop was dipped in the suitable organisms and streaked on the surface of already solidified nutrient agar plates with plant extracts to make a series of parallel non-overlapping streaks. The effects of the plant extracts was expressed as the growth variation found in the Agar plates after incubation at 37° C for 24 hours.

The extracts used for the study were prepared namely,

- Chloroform
- Ethanol
- Petroleum ether

RESULTS AND DISCUSSION

In our present study, the antibacterial activity of six medicinal plants namely, *Asystasia indica* *Asystasia gangetica* *Thunbergia alata* against the tested microorganism (*Salmonella typhi*, *Pseudom aeruginosa*) were evaluated by the disc diffusion and streak plate method using different concentration of solvents. (Mazura and Norin 1993) (Rasooli, and M,r mostofa, (2002)(Puratchi et al 2001) The results showed significantly in all plants samples.

Disc diffusion method

In effect of different solvent extracts of *Asystasia indica* leaf, stem and tuber on the test bacterium *Salmonella typhi* and *Pseudomonas aeruginosa* by disc diffusion method The effect of various solvents namely ethanol, chloroform and petroleum ether also with the standard antibiotics

Among the three solvents extracts used, the ethanolic solvent was found to more effective when compared to other solvents. The plant part tuber has shown higher inhibitory effect among other parts used.

The *Asystasia gangetica*, the results indicated that the leaf have shown better inhibition than other parts such as stem and root by each of the solvents. Antibacterial activity of *Thunbergia alata* showed better results in ethanolic leaf extract as well as stem than root Control plates were prepared for the comparison with the standard antibiotics namely ciprofloxacin.

Streak plate method

The effect of tuber extract in all solvents of *Asystasia indica* Radhika, et al 2002. On the growth of *Slmonella typhi* and *Pseudomonas aeruginosa* by streak plate method The results shows that in control plate excessive growth of both bacteria was observed whereas in the experimental plates gradual increase in concentration of the extract showed decrease growth. That is at 25% concentration moderate growth was observed while in 100% concentration there was no growth. However the solvents of stem and leaf did not show any significant effect when compared to tuber. The streaking of test organism effect on *Asystasia indica* in leaves, stem and root The leaf extract have shown significant effect than stem and root in all solvents of test organism. No growth was occurred in 100% concentration, while excessive growth was showed in control of both organisms.

The streaking effect of ethanolic and chloroform leaf extract of *Asystasia gangetica*, showed that the lower concentration itself inhibited growth of both organism, that is, at 25% concentration itself the growth was less and in 50% only trace growth was noticed on *salmonella typhi* whereas the *Pseudomonas aeruginosa* has shown no growth in 75% concentration on chloroform and petroleum ether solvent The ethanolic and petroleum ether stem extracts of *Asystasia gangetica* have shown gradual decrease of growth of *Salmonella typhi* from 25% concentration as moderate, 50% concentration with trace amount of growth. No growth was occurred in 75% and 100% concentration *Pseudomonas aeruginosa* have shown less growth on 25% concentration of ethanolic and petroleum ether solvents were observed.

The streaking effect of ethanolic leaf extract of *Thunbergia alata* showed excessive growth in 25% concentration less growth in 50% concentration, whereas no growth was occurred in both 75% and 100% concentration.

Salmonella typhi has shown insignificant effect. Chloroform and petroleum ether solvents of leaf extracts have shown identical effect in which trace growth was oserved in 50% and 75% concentration.



Chloroform stem extract of *Thunbergia alata* have shown significant effect on the growth of *Pseudomonas aeruginosa* indicated that trace growth occurred in 50% concentration and no growth was found in 75% and 100% concentration than other solvents.

The results indicate that the rate of inhibition was directly proportional to the concentration of the extracts of most of the experimental plants. At lower concentration inhibition was insignificant whereas at higher concentration the growth inhibition was slightly higher.

DISCUSSION

This experimental result coincides with the results of and (Kasali, A. A., et al 2002. Mundhada and taik 2003.) (Palic et al 2002. Purohit and pruthi 2003) who studied the effect of various solvent extracts of *Hygrophila stricta* and *Peperomia pellucida* and *Schotia brachypetala* respectively on several bacteria. Vijayakumar et al (2003) who studied the effect of various solvent extracts of *Wrightia tinctoria* and *Bauhinia scandens* on various test bacterium also confirms the result of the present study. (Sharma and Verma 1988., Sangeetha et al 1994) who studied the effect of various solvent in extracts of different plant parts of *Curcuma longa* and *Origanum onites* on different bacteria also confirms with the present results. The effect of various solvent extracts of Entrocin Pediatric suspension and edible plants on several bacteria by Goswami et al. (2003) and Alzoreky et al. also agree with the present experimental results.

SUMMARY AND CONCLUSION

The present experimental study concludes with the following:

1. All the experimental plants were found to possess antibacterial properties to some extent especially on few bacteria.
2. Among the plant parts used for antibacterial assay tuber, stem and leaf were found to contain inhibitory effect on the growth of test bacteria.
3. Among the solvents used for extraction, ethanol and chloroform showed more activity than the petroleum ether solvent.
4. With regard to the microbes tested, both organisms were found to be more susceptible to the plant extracts.

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