

## IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF *Tecoma stans* AND *Vitex negundo*

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### ABSTRACT

Plants with antimicrobial activity can serve as potential medicine with lesser side effects. There were many active compounds still present in many medicinal plants which have yet to be discovered. Only the need is to screen them for their potential antimicrobial property. This study aims to evaluate the antimicrobial activities of two medicinal plants – *Tecoma stans* and *Vitex negundo* against *Pseudomonas aeruginosa* and *Aspergillus niger*.

**KEYWORDS:** Antimicrobial Activity, *Tecoma stans*, *Vitex negundo*, *Pseudomonas aeruginosa*

Natural products of either plants or animals were used in human civilization as the main source of medicine from the ancient period. Plants and their extracts were used as traditional herbal medicine for a long time by ancient peoples to cure all kinds of diseases (Sowjanya et. al., 2013). Secondary metabolites of various medicinal plants are rich source of medicine and developing rapidly in practice challenging the modern medicine (Lutterodt et. al., 1999). In developing countries the traditional herbal medicines is used for primary treatment and considered as an important health care system. Various parts of plants such as leaves, roots, seeds, fruits, flowers etc were used traditionally and were found to have potential antimicrobial properties (Sowjanya et. al., 2013). Plants with antimicrobial activity can serve as potential medicine with lesser side effects. There were many active compounds still present in many medicinal plants which have yet to be discovered. Only the need is to screen them for their potential antimicrobial property. This study aims to evaluate the antimicrobial activities of two medicinal plants – *Tecoma stans* and *Vitex negundo* against *Pseudomonas aeruginosa* and *Aspergillus niger*.

*Tecoma stans* belongs to family Bignoniaceae, is a native plant of America. It is a large shrub, much branched with 10-25cm long compound leaves. Flowers are bright yellow in color, tubular in shape and born in clusters. Its flower gives an attractive look to the plants and thus in homes, gardens, road sides etc. it is planted as show plant. Fruits are large, elongated with flat seeds. The plant was used traditionally in Mexico and Central America for diabetic and urinary disorders. Leaves of plant contain many biologically active chemicals such as various types of alkaloids, flavinoids, steroids, tannins,

phytosterols, hydrocarbons, resins, volatile oil, glycosides etc. (Raju et. al., 2011). Various primary and secondary metabolites such as sugar, alkaloids, triterpenoids and phenolics were identified in the whole plant of *T. stans* (Dohnal, 1976). Literature survey reveals that *T. stans* possess various bio active compounds exhibiting antioxidant, antibacterial and antimicrobial activities (Karou et. al., 2006). It shows antimicrobial property against many pathogenic bacteria such as *Klebsiella pneumonia*, *Staphylococcus* species, *Salmonella* species, *Vibrio parahemolyticus* (Senthilkumar et. al., 2010) etc. Its leave and stem extract contain significant antibacterial property against some species of *Bacillus*, *Staphylococcus*, *Pseudomonas* (Salem et. al., 2013) etc. It also contains a potent antifungal property against *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigates* and *Fusarium solani* (Javid et. al., 2015). Tecomine isolated from *T. stans* possess potential hypoglycemic effect in animals (Amad et. al., 2012, Raju et. al., 2011).

*V. negundo*, commonly known as nirgundi belongs to family Verbenaceae, is a woody, large aromatic shrub bearing tri or penta foliate leaves. Its flower is bluish purple in color. It is native to tropical Eastern and Southern Africa and Asia. Number of active phytochemical compounds was found in plant extract of nirgundi. It has been found that *V. negundo* serve as an important medicinal plant and used traditionally to cure various diseases in many countries in Asia (Chowdhary et. al., 2010). It contains many types of phytochemicals compounds such as volatile oils, flainoids, terpens, steroids (Banerji et. al., 1988, Dayal R. and Singh V., 2000, Maurya et. al., 2007) etc.

## MATERIALS AND METHODS

### Collection of Plant Material

Fresh plant leaves of *T. stans* and *V. negundo* were collected from college campus, Bhilai. The leaves were washed with tap water to remove dusts, then air dried under shade at room temperature. The leaves were then powdered with the help of mechanical mills.

### Phytochemical Screening

Standard screening test of water extract of leaves of *T. stans* and *V. negundo* was carried out for various plant constituents. The water extract of leaves of both the plants were screened for the presence and absence of important phytochemical such as alkaloids, carbohydrates, cardiac glycoside, flavinoids, phenols, amino acids and proteins, saponins, tannins, terpenoids and resins using standard procedure [Harborne J.B., 1998 & Khandelwal K.R., 2007].

### Estimation of Alkaloids

1 ml leaf extract was treated with 3-5 drops of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

### Estimation of Carbohydrates

1 ml of leaf extract was treated with 3-5 drops of Molish reagent. To this 1ml of concentrated sulphuric acid was added from the side of the test tube. The mixture was allowed to stand for 2-3 min. Formation of red or dull violet color at the interface of the two layers indicate the presence of carbohydrate.

### Estimation of Cardiac Glycosidase

1 ml of leaf extract was treated with 1 ml of glacial acetic acid and 2-3 drops of 5% ferric chloride solution. Then 0.5 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added to the mixture. Formation of brown ring at the interface shows presence of cardiac glycoside.

### Estimation of Flavonoids

1 ml of leaf extract was treated with 3-5 drops of 20% NaOH solution. Intense yellow color was formed which become colorless on adding 0.5 ml of dilute HCl. It indicates the presence of flavinoids.

### Estimation of Phenols

1 ml of leaf extract was treated with 5-6 drops of 5% aqueous ferric chloride solution. Deep blue or black color formed indicating the presence of phenols.

### Estimation of Amino Acid and Proteins

1 ml of leaf extract was treated with 2-5 drops of Ninhydrine solution. The mixture was kept in boiling water for 1-2 minutes. Purple color was found indicating the presence of amino acid and proteins.

### Estimation of Saponins

To 1 ml of leaf extract 5 ml of distilled water was added and shaken vigorously. Foam was formed and persists for 10-15minutes confirming the presence of saponins.

### Estimation of Tannins

1 ml of leaf extract was treated with 1 ml of 10% alcoholic ferric chloride solution. Formation of blue or green color confirms the presence of tannins.

### Estimation of Terpenoids

1 ml of leaf extract was treated with 0.5 ml of chloroform along with 3-5 drops of concentrated sulphuric acid. Reddish brown precipitate immediately produced confirming the presence of terpenoids.

### Estimation of Resins

5 ml distilled water was added to 1 ml of leaf extract. Turbidity of mixture indicates the positive result.

### Extract Preparation

30gm of the dried powdered leaves of both plants were soaked in 300ml of ethanol, chloroform and water for week, shaking 2 times in a day and then filtered. All the extracts were stored in sterile flask at room temperature until screened.

### Isolation of Bacteria

*P. aeruginosa* were isolated from sample by serial dilution method in Kings Agar B medium and were identified using protocol of Bergey's manual of determinative bacteriology.

### Isolation of Fungi

Fungal strains *A. niger* was isolated and identified and used for antifungal activities.

### Antimicrobial Bioassay

The three different solvents were subjected to antibacterial and antifungal activities against *P. aeruginosa* and *A. niger* respectively by Agar Well Diffusion Method described by Bauer *et al*, 1966. For these 24hrs culture of bacteria and 42hrs culture of fungi were prepared, separately in broth media by taking organism from their respective agar medium. Inoculums were prepared by diluting bacteria and fungi separately in distilled water. These suspensions of bacteria and fungi were uniformly spread on nutrient agar plate and potato dextrose agar plate respectively and left for 15-20 minutes. In three side of each plate 6mm diameter of well were dug. In one well water extract of test sample were taken in concentration 400µg/ml. in another well ethanol extract of the test samples were taken and in third well chloroform extract of the test samples were taken

separately for bacteria and fungi. The plates of bacteria were then allowed to incubate at 37°C for 24hrs and fungal plates were incubated at 30°C for 3 days. After 24 hrs antibacterial activities were measured by measuring the diameter of zone of inhibition and after 3 days the fungal growth were observed by measuring zone of inhibition diameter.

## RESULTS AND DISCUSSION

### Phytochemical Screening

The preliminary phytochemical screening of aqueous leaf extract of *T. stans* and *V. negundo* was presented in Table. 1. Except saponins all the other tested compounds were found to be present in the water extract of leaf of *T. stans*. In *V. negundo* saponin and phenol give negative test and rest of the other compounds were found to give positive result.

**Table 1: Phytochemicals analysis of water extracts of leaves of *T. stans* and *V. negundo*.**

S. No.	Test	<i>Tecoma stans</i>	<i>Vitex negundo</i>
1.	Alkaloids	+	+
2.	Carbohydrate	+	+
3.	Cardiac glycoside	+	+
4.	Flavionoid	+	+
5.	Saponin	-	-
6.	Tannin	+	+
7.	Resin	+	+
8.	Terpenoids	+	+
9.	Amino acid and protein	+	+
10.	Phenol	+	-

### Antimicrobial Bioassay

Antimicrobial activity of water, ethanol and chloroform extracts of *T.stans* and *V.nirgudo* were

evaluated by measuring the diameter of zones and the result was tabulated in Table 2.

**Table 2: Antibacterial and antifungal activity of *T. stans* and *V. negundo***

S.No.	Leaf extract	Zone of inhibition in mm			
		<i>T.stans</i>		<i>V.negundo</i>	
		<i>P.aeruginosa</i>	<i>A. niger</i>	<i>P.aeruginosa</i>	<i>A. niger</i>
1.	Water	13	11	16	19
2.	Ethanol	18	16	20	22
3.	Chloroform	15	14	19	17

Both the tested microorganisms were found susceptible to the leave extract of *T. stans* and *V. negundo*. but vary for different solvents. Our result reveals that antimicrobial activity of water, ethanol and

chloroform extract of *V. negundo* shows significant higher inhibitory activity for both bacteria and fungi as compared to *T. stans*. For *A. niger* the zone of inhibition of water extract was found to be 11mm, that of ethanol and

chloroform extract of *T. stans* were found to be 16mm and 14mm respectively. For *P. aeruginosa* the zone of inhibition of water, ethanol and chloroform extract of *T. stans* were found to be 13mm, 18mm and 15mm respectively. Similarly the zone of inhibition of different extract of *V. negundo* was different for both *P. aeruginosa* and *A. niger*. For *A. niger* the zone of inhibition was 19mm, 22mm and 17mm by water, ethanol and chloroform extract respectively. For *P. aeruginosa*, it was 16mm, 20mm and 19 mm by water, ethanol and chloroform extract respectively. The zone of inhibition for both bacteria and fungi was observed maximum in ethanol extract and minimum in water extract except for fungi in *V. negundo* in which the minimum zone of inhibition was observed in chloroform extract.

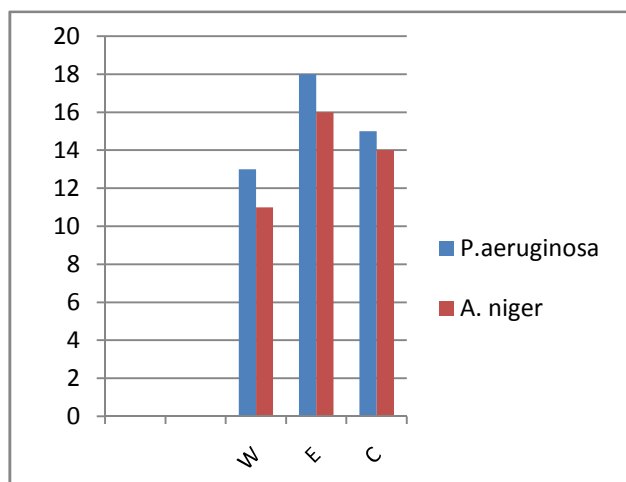


Figure 1: Zone of inhibition in mm by leaf extract of *T. stans*

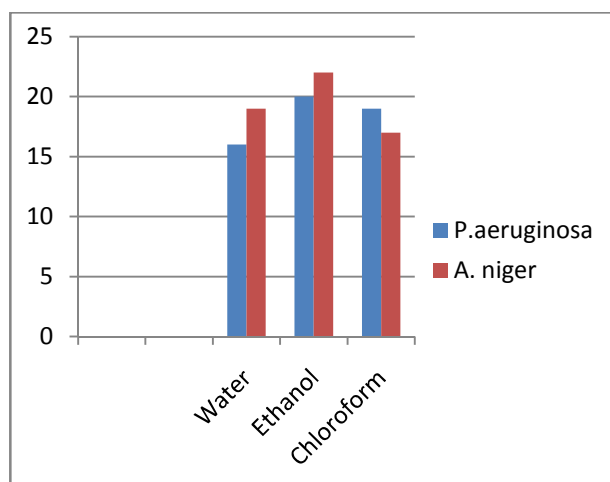


Figure 2: Zone of inhibition in mm by leaf extract of *Vitex negundo*

The present investigation has shown that the ethanol extract of both the plants have active phytochemical which can inhibit the growth of pathogenic bacteria and fungi (Bauer et. al., 1966, Harborne, 1998). Most significant antimicrobial activity of ethanol extract was due to the active compound of *T. stans* and *V. negundo*. The presence of important constituent such as tannins, glycosides etc. are responsible for antimicrobial activity. The ethanol leaf extract of *V. negundo* showed the highest antimicrobial activity against *A. niger*. The chloroform extract of *V. negundo* show highest activity against *P. aeruginosa*. But water extract of *V. negundo* show maximum antimicrobial activity against *A. niger*. Highest antimicrobial activity against *P. aeruginosa* by methanol and chloroform leaf extract (Silver, 1993). Similarly inhibition of growth of all the tested microbes by methanol and chloroform extract of *T. stans* was reported. The highest antibacterial activity of extracts against the growth of *S. marcescens* was found in methanol and chloroform. Thus the plants are useful for production of antibacterial drugs for the treatment of infection caused by bacteria. Although both the plant contain important compounds with antimicrobial activities against wide range of pathogenic microbes, but their aqueous solution did not exhibit much zone of inhibition for bacteria and fungi. Presence of alkaloids in the plants have been shown to possess an antimicrobial activities (Bauer et. al., 1966, Salem et. al., 2013). Highest antimicrobial activities were observed in the methanolic extract of leaves of *T. stans* against various tested bacteria (Muthu et. al., 2012). Significant antibacterial activity of leaf extract of *V. negundo* against *Staphylococcus aureus* was evaluated (Sowjanya et.al., 2013)(Figure 1& 2).

## CONCLUSION

Both the tested plant showed inhibition of growth of bacteria and fungi in their respective media. The present study deals with the antimicrobial properties of leaves of *Tecoma stans* and *Vitex negundo* with water, ethanol and chloroform extract by agar well diffusion method. It was found that ethanol and chloroform leaf extracts of *Tecoma stans* exhibit significant antibacterial and antifungal activity when compared to water extract. The ethanol leaf extract showed the highest activity against *P. aeruginosa*. But only the ethanol leaf extract of *Vitex negundo* exhibit strong antibacterial and antifungal activity. The results of present phytochemical and antimicrobial study of both the plants revealed that these

plants must be exploiting for natural flora for the commercial production of medicines. Our observation supports the long term antimicrobial properties of these plants. The studies on the leaves of *Tecoma stans* and *Vitex nirgundo* plant showed good potential to utilize these plant more and more for commercial purpose. Now a day's interest has been increased in plant derived medicine since it is considered that herbal medicine is cheaper and safer than synthetic drugs which is costly and possesses side effects. Hence plants of medicinal uses such as *Tecoma stans* and *Vitex negundo* must be needed to screen more and more to obtain promising biologically active compounds. And also since there is continuous development in resistant microbial pathogens so there is need for development of new drugs also (Silver, 1993).

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