



STUDY OF ANTIMICROBIAL ACTIVITY, PHYTOCHEMICAL SCREENING & TLC OF *Citrus limon*, *Syzygium cumini*, *Vitex negundo* & *Tinospora cordifolia* AND THEIR PRODUCTS

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ABSTRACT

The antimicrobial activity of various plants like *Citrus limon* (Lemon), *Syzygium cumini* (Jamun), *Vitex negundo* (Nagod) & *Tinospora cordifolia* (Galo) were studied and were tested on test organisms with the help of agar well diffusion method. Leaves of *Vitex negundo* & *Tinospora cordifolia*, juice of *Citrus limon* and fruit of *Syzygium cumini* has been taken for the study purpose. Almost all the plants showed zone of inhibition against the tested organisms like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* & *Proteus vulgaris* in high or less effectively but *Vitex negundo* (Nagod), *Citrus limon* (Lemon) & *Syzygium cumini* (Jamun) shows better results among all the plants extracts. Phytochemical screening as well as TLC were also studied of all the plant extracts. Hence, the leaf extracts of all above plants can also be used for further investigation for determination of therapeutic potential.

KEYWORDS: Antimicrobial activity, Agar well diffusion, , Phytochemical screening, TLC, Plant extracts



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INTRODUCTION

Plants have been a rich source of natural products for maintaining human health. Existence of human kind is impossible without plants. It has been found that plants have potential antibiotics for the cure of diseases by antimicrobials. Traditionally crude plants have been used as a medicine. This property is because of bioactive compounds synthesized during secondary metabolism in plants. Although this generation is the evidence of great success in development of technology, science, and medicine, but somewhere we failed to control the dramatic spread of infection. WHO stated that the infectious diseases remains the second leading cause of death worldwide (WHO)¹ The rise in search of new drugs from the plants is because of the microorganisms causing serious health problems. Day by day many of the microorganisms are becoming resistance to the most of the chemically synthesized drugs. So it become a reason to find newer drugs which are effective against those diseases and as well as chemically balanced, effective & least injurious with no or less side effects. The following plants has been used for studying Antimicrobial activity. Plants like *Citrus limon* (Lemon), *Syzygium cumini* (Jamun), *Vitex negundo* (Nagod) & *Tinospora cordifolia* (Galo) are rich in phytochemicals and nutrients and can be used as remedies against various diseases. *Syzygium cumini* is belonging to the family Myrtaceae. Large trees cultivated throughout India for the edible fruits (Black Plum) and are reported to contain vitamin C, gallic acid, anthocyanins, includes cyanidin, petunidin, malvidinglucoside and other components.² *Syzygium cumini* is a medicinal plant, whose parts were pharmacologically proved to posses hypoglycaemic, anti-HIV activity and anti-diarrhea effects.^{3,4,5,6} Leaves and barks of *Syzygium cumini* have anti-inflammatory activity.⁷ Leaves have been also used in traditional medicine as a remedy for diabetes mellitus in many countries. *Vitex negundo* belongs to the family Lamiaceae. The principal constituents of the leaf juice are casticin, isoorientin, chrysophenol D, luteolin, p-hydroxybenzoic acid and D-fructose. The main constituents of the oil are sabinene, linalool, terpinen-4-ol, β -6-caryophyllene, α -guaiene and globulol constituting 61.8% of the oil. Lemons are a rich source of vitamin C, providing 64% of the Daily Value in a 100 g serving. Lemons contain numerous phytochemicals, including polyphenols, terpenes. Lemon oil may be used in aromatherapy. Lemon oil aroma does not influence the human immune system,⁸ but may contribute to relaxation.⁹ *Tinospora cordifolia* is an important drug of Indian systems of medicine and used in medicines since times immemorial. The drug is well known Indian bitter and prescribed in fevers, diabetes, dyspepsia, jaundice, urinary problems, skin diseases and chronic diarrhoea and dysentery. It has been also indicated useful in the treatment of heart disease, leprosy, and helmenthiasis. The starch obtained from the stem is highly nutritive and digestive and used in many diseases.¹² It has been reported to mediate its anti-diabetic potential through myriad of biologically active phytoconstituents isolated from different parts of plant, including alkaloids, cardiac

glycosides, flavanoids, saponins and steroids.¹⁰ These compounds have been reported to encompass different target activities in diabetic conditions, thus enabling the potential application in experimental and clinical research. The gold standard drug for the treatment of Parkinson's disease is L-DOPA, but various studies have proved that the treatment with L-DOPA leads to the death of surviving dopaminergic neurons in the CNS. The co-administration of *Tinospora cordifolia* crude powder protected the dopaminergic neurons when compared with Sham operated control group. The treatment with *Tinospora cordifolia* crude powder could reduce the toxicities of L-DOPA therapy for Parkinson's disease.¹¹ However though there are many other plants which have great medicinal values, less studies have been carried out to evaluate the antimicrobial activity of all the above plants and as well as the study of their phytochemical screening and thin layer chromatography of all the above *Citrus limon*, *Syzygium cumini*, *Vitex negundo* & *Tinospora cordifolia*

MATERIALS AND METHODS

Sample collection

All the plant *Citrus limon*, *Syzygium cumini*, *Vitex negundo* & *Tinospora cordifolia* leaves, juice and fruit of the plants were collected from different places of Valsad region, Gujarat. The plant materials were washed under tap water and then dried in hot air oven and fine powder was prepared.

Sample extraction

After making fine powder of the plants materials 1gm was vortexed with 5ml of the methanol and filtered it to obtain the filtrate is kept at room temperature for the evaporation of the methanol for 24 hours and then they were used for the further study

Bacterial cultures

To study the antibacterial activity, the microbial pure cultures obtained from the Laboratory of Microbiology Department of Dolat-Usha Institute of Applied Sciences and Dhiru-Sarla Institute of Management & Commerce College, Valsad. Microorganisms were maintained on nutrient agar slants at 4°C and sub-cultured every month. The microbial strain studied were gram positive cocci *Staphylococcus aureus*, gram positive rod *Bacillus subtilis*, and gram negative rods *Escherichia coli*, *Pseudomonas aeruginosa* & *Proteus vulgaris*.

Antimicrobial activity

Antimicrobial activity of plant extract were carried out using agar well diffusion method.¹² Extracts of leaves were delivered into wells which were formed in the nutrient agar plates containing bacterial inoculum. Then the nutrient agar plates were incubated at 37°C for 24 hrs. The presence of microbial zone of inhibition indicates the antimicrobial action, measured in millimetre (mm). The zones were measured with the help of zone meter.

Phytochemical screening

Phytochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants.^{13,14} The test for Terpenes,

Flavanoids, Saponins, Cardiac glycosides, Protein, Carbohydrate, was carried out for all the plant extracts. The procedures of all the above tests were mentioned in the table no. 1.

Table 1
Process and standard observation of all tests of phytochemical screening

| Sr. No. | Test | Procedure | Standard observation |
|---------|--------------------|--|------------------------------------|
| 1 | Terpenes | 5ml extract+2ml chloroform+3ml conc. H ₂ SO ₄ | Reddish brown ring |
| 2 | Flavonoids | Small amount of extract+few drop of conc. HCl | Immediate development of red color |
| 3 | Saponins | Extract in powder form+10ml of distilled water. Shaken well for 15minutes | Formation of foam |
| 4 | Cardiac glycosides | 2ml glacial acetic acid containing 1 drop of FeCl ₃ +1ml conc. H ₂ SO ₄ | Appearance of brown ring |
| 5 | Protein | 3ml extract+4% NaOH+few drops of 1% CuSO ₄ | Formation of violet or pink colour |
| 6 | Carbohydrate | 2-3ml of extract+2 drops of α naphthol, shake & add conc. H ₂ SO ₄ from side of test tube | Violet ring is formed |

Thin layer chromatography (TLC)

Thin layer chromatography (TLC) is a technique used for the separation, identification and estimation of single or mixture of components present in various extracts. TLC Plates- Used for separation were precoated silica gel. It acted as stationary phase. Sample application- The extracts were spotted with the help of capillary tube just 1cm above its bottom. Selection of mobile phase- Solvent mixture was selected on the basis of the phyto constituents present in each extract. Chloroform: Methanol (15 : 5) was used as mobile phase. Iodine crystals was used as developing agent.

RESULTS AND DISCUSSIONS

The results of antimicrobial activity, phytochemical screening and TLC of all plants extracts were summarized in Table 2,3, & 4, respectively. All the plants were extracted in methanol. *Vitex negundo* showed zone of inhibition against *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus* & *Bacillus subtilis* and did not show zone of inhibition against *Escherichia coli*. Phytochemical screening showed positive results in tests for terpenes, cardiac glycosides & carbohydrate and showed absence in tests for saponin, protein, & flavanoids. The TLC results showed 4 spots that means 4 components were separated. Our results of antimicrobial activity on some of the tested organisms is similar to the earlier studied. And our results of phytochemical screening were similar to the results carried out earlier.¹⁵ The *Citrus limon* showed zone of inhibition against *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris* & *Pseudomonas*

aeruginosa and does not showed any activity against *Staphylococcus aureus*. Phytochemical screening showed positive results in tests for carbohydrate and showed absence in tests for terpenes, flavanoids, cardiac glycosides, tannin and phenolic compounds, saponin & protein. The TLC results did not show any spots that means not a single components had been separated. Our results of antimicrobial activity is almost similar in all tested organisms but the results of phytochemical screening is contradictory as compared with the results of antimicrobial activity and phytochemical screening carried out earlier.¹⁶ The *Syzgium cumini* showed zone of inhibition against *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris* & *Pseudomonas aeruginosa* and does not showed any activity against *Staphylococcus aureus*. Phytochemical screening showed positive results in tests for terpenes and carbohydrate and showed absence in tests for Saponins, cardiac glycosides, protein, flavanoids & tannin and phenolic compound. The TLC results showed 3 spots. Our results of antimicrobial activity is good as compared with the results of¹⁷ and the results of phytochemical screening is contradictory as compared with the earlier study.¹⁸ The *Tinospora cordifolia* showed zone of inhibition against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* & *Proteus vulgaris*. Phytochemical screening showed positive results in tests for terpenes, saponins, cardiac glycosides & carbohydrates and showed absence in tests for flavanoids, protein & tannin and phenolic compounds. The TLC results showed 1 spot. We got better results of antimicrobial activity and similar results of phytochemical screening as compared with the earlier analysis.¹⁹

Table 2
Inhibition zones of solvent extracts of all plants against Gram-positive and Gram negative bacteria

| Sr. No. | Plants used | Organisms | | | | |
|---------|-----------------------------|-------------------------|-------------------------------|-------------------------|------------------------------|--------------------------|
| | | <i>Escherichia coli</i> | <i>Pseudomonas aeruginosa</i> | <i>Proteus vulgaris</i> | <i>Staphylococcus aureus</i> | <i>Bacillus subtilis</i> |
| 1 | <i>Vitex negundo</i> | - | 20mm | 17mm | 11mm | 18mm |
| 2 | <i>Citrus limon</i> | 16mm | 16mm | 14mm | - | 18mm |
| 3 | <i>Syzygium cumini</i> | 15mm | 22mm | 11mm | - | 13mm |
| 4 | <i>Tinospora cordifolia</i> | 11mm | 13mm | 10mm | 15mm | 10mm |

Table 3
Phytochemical screening of plant extracts

| Sr. No. | Test | Observation | | | |
|---------|--------------------|----------------------|---------------------|------------------------|-----------------------------|
| | | <i>Vitex negundo</i> | <i>Citrus limon</i> | <i>Syzygium cumini</i> | <i>Tinospora cordifolia</i> |
| 1 | Terpenes | + | - | + | + |
| 2 | Flavonoids | - | - | - | - |
| 3 | Saponins | - | - | - | + |
| 4 | Cardiac glycosides | + | - | - | + |
| 5 | Protein | - | - | - | - |
| 6 | Carbohydrate | + | + | + | + |

Table 4
TLC results of all plants extracts

| Sr.no. | Plants | No. of spots obtained on TLC sheet | Rf values |
|--------|-----------------------------|------------------------------------|------------------------|
| 1 | <i>Syzygium cumini</i> | 3 | 0.07, 0.2, 0.44 |
| 2 | <i>Vitex negundo</i> | 4 | 0.23, 0.56, 0.84, 0.92 |
| 3 | <i>Citrus limon</i> | - | - |
| 4 | <i>Tinospora cordifolia</i> | 1 | 0.2 |

CONCLUSION

The plants have been extensively studied in terms of pharmacological activity of its major components. In recent years, emphasis of research has been on utilizing traditional medicines that have long and proven history of treating various diseases. From the above observation and results it can be concluded that all the

above plants extract need to be further so that they may be used as a future drug to prove its efficacy as a preventive and therapeutic agent against the pathogens.

CONFLICT OF INTEREST

Conflict of interest declared none.

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