



## 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)<sup>1</sup> 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.<sup>2</sup> *Syzygium cumini* is a medicinal plant, whose parts were pharmacologically proved to posses hypoglycaemic, anti-HIV activity and anti-diarrhea effects.<sup>3,4,5,6</sup> Leaves and barks of *Syzygium cumini* have anti-inflammatory activity.<sup>7</sup> 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,  $\beta$ -6-caryophyllene,  $\alpha$ -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,<sup>8</sup> but may contribute to relaxation.<sup>9</sup> *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.<sup>12</sup> 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.<sup>10</sup> 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.<sup>11</sup> 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.<sup>12</sup> 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.<sup>13,14</sup> 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 <sub>2</sub> SO <sub>4</sub>	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 <sub>3</sub> +1ml conc. H <sub>2</sub> SO <sub>4</sub>	Appearance of brown ring
5	Protein	3ml extract+4% NaOH+few drops of 1% CuSO <sub>4</sub>	Formation of violet or pink colour
6	Carbohydrate	2-3ml of extract+2 drops of $\alpha$ naphthol, shake & add conc. H <sub>2</sub> SO <sub>4</sub> 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.<sup>15</sup> 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.<sup>16</sup> 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 of<sup>17</sup> and the results of phytochemical screening is contradictory as compared with the earlier study.<sup>18</sup> 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.<sup>19</sup>

**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.

## REFERENCES

1. Abdallah EM. Plants: An alternative source for antimicrobials.
2. Martinez SB, Del Valle MJ. Storage stability and sensory quality of duhat (*Syzygium cumini* Linn.) anthocyanins as food colorant. UP Home Economic Journal. 1981;9(1):1981.
3. Bhuiyan MS, Younus Mia M, Rashid MA. Antibacterial principles of the seeds of *Eugenia jambolana*. Bangladesh J. Bot. 1996;25(2):239-41.
4. Kusumoto IT, Nakabayashi T, Kida H, Miyashiro H, Hattori M, Namba T, Shimotohno K. Screening of various plant extracts used in ayurvedic medicine for inhibitory effects on human immunodeficiency virus type 1 (HIV-1) protease. Phytotherapy Research. 1995 May 1;9(3):180-4.
5. Kumar A, Padmanabhan N, Krishnan MR. Central nervous system activity of *Syzygium cumini* seed. Pakistan journal of nutrition. 2007;6(6):698-700.
6. Ravi K, Sekar DS, Subramanian S. Hypoglycemic activity of inorganic constituents in *Eugenia jambolana* seed on streptozotocin-induced diabetes in rats. Biological trace element research. 2004 Jun 1;99(1-3):145-55.
7. Muruganandan S, Srinivasan K, Chandra S, Tandan SK, Lal J, Raviprakash V. Anti-inflammatory activity of *Syzygium cumini* bark. Fitoterapia. 2001 May 31;72(4):369-75.

8. Rauf A, Uddin G, Ali J. Phytochemical analysis and radical scavenging profile of juices of *Citrus sinensis*, *Citrus anrantifolia*, and *Citrus limonum*. *Organic and medicinal chemistry letters*. 2014 Dec 1;4(1):1-3.
9. Penniston KL, Nakada SY, Holmes RP, Assimos DG. Quantitative assessment of citric acid in lemon juice, lime juice, and commercially-available fruit juice products. *Journal of Endourology*. 2008 Mar 1;22(3):567-70.
10. Gaur LB, Singh SP, Gaur SC, Bornare SS, Chavan AS, Kumar S, Ram M.
11. A Basic Information, Cultivation and Medicinal Use of *Tinospora cordifolia*. *Pop. Kheti*. 2014; 2(3):188-192.
12. Sharma R, Amin H, Prajapati PK. Antidiabetic claims of *Tinospora cordifolia* (Willd.) Miers: critical appraisal and role in therapy. *Asian Pacific Journal of Tropical Biomedicine*. 2015 Jan 31;5(1):68-78.
13. Antony S, DebRoy P, Vadivelan R, Jaysankar K, Vikram M, Nandini S, Sundeep M, Elango K, Suresh B. Amelioration of CNS Toxicities of L-Dopa in Experimental Models of Parkinson's disease by Concurrent Treatment with *Tinospora cordifolia*. *Hygeia: Journal for Drugs and Medicines*. 2010 Mar;2(1):28-37. 13 Rajendran NK, Ramakrishnan J. In vitro evaluation of antimicrobial activity of crude extracts of medicinal plants against multi drug resistant pathogens. *Biyoloji Bilimleri Araştırma Dergisi*. 2009;2:97-101.
14. Khatak S, Naagar J, Gupta A, Malik DK. Antimicrobial activity of *Vitex negundo* against pathogenic bacteria. *J. Pharm. Res*. 2014 Feb):91-2.
15. SARA VANASINGH D, RAMAMURTHY D. BIOACTIVITY STUDIES OF EXTRACTS FROM *TRIBULUS TERRESTRIS*. *Int. J. Curr. Res. Chem. Pharm. Sci*. 2016;3(1):40-9.
16. Okeke MI, Okoli AS, Eze EN, Ekwume GC, Okosa EU, Iroegbu CU. Antibacterial activity of *Citrus limonum* fruit juice extract. *Pak. J. Pharm. Sci*. 2015 Sep 1;28(5):1567-71.
17. Kaneria M, Baravalia Y, Vaghasiya Y, Chanda S. Determination of antibacterial and antioxidant potential of some medicinal plants from Saurashtra region, India. *Indian journal of pharmaceutical sciences*. 2009 Jul;71(4):406-18. Kalaiselvi K, Selvi S. PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *CUCURBITA PEPO* LEAVES.
18. Sandhu A, Bhardwaj N, Gupta R, Menon V. Antimicrobial Activity and Photochemical Screening of *Tinospora Cordifolia* and *Euphorbia Hirta*.

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