

Vol 3 Issue 3 Sept 2013

Impact Factor : 1.2018 (GISI)

ISSN No :2231-5063

Monthly Multidisciplinary  
Research Journal

*Golden Research  
Thoughts*

Chief Editor  
Dr.Tukaram Narayan Shinde

Publisher  
Mrs.Laxmi Ashok Yakkaldevi

Associate Editor  
Dr.Rajani Dalvi

Honorary  
Mr.Ashok Yakkaldevi

**IMPACT FACTOR : 0.2105**

**Welcome to ISRJ**

**RNI MAHMUL/2011/38595**

**ISSN No.2230-7850**

Indian Streams Research Journal is a multidisciplinary research journal, published monthly in English, Hindi & Marathi Language. All research papers submitted to the journal will be double - blind peer reviewed referred by members of the editorial Board readers will include investigator in universities, research institutes government and industry with research interest in the general subjects.

### ***International Advisory Board***

Flávio de São Pedro Filho Federal University of Rondonia, Brazil	Mohammad Hailat Dept. of Mathematical Sciences, University of South Carolina Aiken, Aiken SC 29801	Hasan Baktir English Language and Literature Department, Kayseri
Kamani Perera Regional Centre For Strategic Studies, Sri Lanka	Abdullah Sabbagh Engineering Studies, Sydney	Ghayoor Abbas Chotana Department of Chemistry, Lahore University of Management Sciences [ PK ]
Janaki Sinnasamy Librarian, University of Malaya [ Malaysia ]	Catalina Neculai University of Coventry, UK	Anna Maria Constantinovici AL. I. Cuza University, Romania
Romona Mihaila Spiru Haret University, Romania	Ecaterina Patrascu Spiru Haret University, Bucharest	Horia Patrascu Spiru Haret University, Bucharest, Romania
Delia Serbescu Spiru Haret University, Bucharest, Romania	Loredana Bosca Spiru Haret University, Romania	Ilie Pinteau, Spiru Haret University, Romania
Anurag Misra DBS College, Kanpur	Fabricio Moraes de Almeida Federal University of Rondonia, Brazil	Xiaohua Yang PhD, USA
Titus Pop	George - Calin SERITAN Postdoctoral Researcher	Nawab Ali Khan College of Business Administration

### ***Editorial Board***

Pratap Vyamktrao Naikwade ASP College Devrukh,Ratnagiri,MS India	Iresh Swami Ex - VC. Solapur University, Solapur	Rajendra Shendge Director, B.C.U.D. Solapur University, Solapur
R. R. Patil Head Geology Department Solapur University, Solapur	N.S. Dhaygude Ex. Prin. Dayanand College, Solapur	R. R. Yaliker Director Managment Institute, Solapur
Rama Bhosale Prin. and Jt. Director Higher Education, Panvel	Narendra Kadu Jt. Director Higher Education, Pune	Umesh Rajderkar Head Humanities & Social Science YCMOU, Nashik
Salve R. N. Department of Sociology, Shivaji University, Kolhapur	K. M. Bhandarkar Praful Patel College of Education, Gondia	S. R. Pandya Head Education Dept. Mumbai University, Mumbai
Govind P. Shinde Bharati Vidyapeeth School of Distance Education Center, Navi Mumbai	Sonal Singh Vikram University, Ujjain	Alka Darshan Shrivastava Shaskiya Snatkottar Mahavidyalaya, Dhar
Chakane Sanjay Dnyaneshwar Arts, Science & Commerce College, Indapur, Pune	G. P. Patankar S. D. M. Degree College, Honavar, Karnataka	Rahul Shriram Sudke Devi Ahilya Vishwavidyalaya, Indore
Awadhesh Kumar Shirotriya Secretary, Play India Play (Trust),Meerut	Maj. S. Bakhtiar Choudhary Director,Hyderabad AP India.	S.KANNAN Ph.D , Annamalai University,TN
	S.Parvathi Devi Ph.D.-University of Allahabad	Satish Kumar Kalhotra
	Sonal Singh	

**Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India  
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.isrj.net**



**GRT** ANALYSIS THE ANTIBACTERIAL AND ANTIOXIDANT  
ACTIVITY OF SELECTED MEDICINAL PLANTS-  
( *Lippia Nodiflora L. And Vitex Negundo L.* )



**Thamaraiselvi<sup>1</sup>, Ezhilarasu<sup>1</sup> and Gajendiran<sup>2</sup>**

<sup>1</sup>P G and Research department of Microbiology, Selvamm arts and science college, Namakkal, Tamilnadu, India

<sup>2</sup>Research scholar Department of Microbiology, Annamalai University, Chidambaram, Tamilnadu, India

**Abstract:**Plants have proved to be significant natural resources for medicines; documentation of their use in medicine originates from ancient times. Ethnobotanical and ubiquitous plants provide a rich resource for natural drug research and development. In the present investigation, the phytochemical and DPPH activity were high in *Lippia nodiflora* (Linn.) while antibacterial activity and H<sub>2</sub>O<sub>2</sub> scavenging activity were maximum in *Vitex negundo* (Linn.) The methanol extract is best solvent for phytochemical and antimicrobial activity of both selected plant against test pathogens. Both plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant; such as phenols, terpenoids, alkaloids and flavonoids. Synergism between plant extract and synthetic antibiotics can develop standardization of herbal medicine for treatment and prevention of infectious diseases.

**Keywords:**Medicinal plant, Phytochemical, Antibacterial, Antioxidants.

**INTRODUCTION:**

In India the use of different parts of several medicinal plants to cure specific elements has been in vogue from ancient times. The indigenous system of medicine namely ayurvedic, siddha and unani have been in existence for several centuries. In homeopathy system 70% of the medicines are prepared from plants. As homeopathy originated in Europe naturally majority of the drugs prepared from plants are of exotic origin (Jeyachandran, et al., 2010 and Rajasekar et al.2007) This plant was widely used by all sections of the society whether directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine (Bhagwati, 2003). In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems (Ayurveda, Siddha and Unani) (Dahanukar et al 2000).

Plants have been used in developing countries as alternative treatments for curing various diseases. In recent years, there has been an increasing awareness about the importance of medicinal plants. Most of the medicinal plant parts are used as raw drugs and they possess varied medicinal properties. Medicinal plants represent a rich source of antimicrobial agent (Mahesh 2008). The world health organization recently compiled an inventory of more than 20,000 species of medicinal plants. Plants provide basic raw materials for the indigenous pharmaceutical industries such as medicinal, cosmetic, perfumery and food (Cowan, 1999 and Nikaido 1998). Nowadays, the development of resistance by a pathogen to many of the commonly used antibiotics provides an impetus for further attempts to search for new

antimicrobial agents (Ali-Shtayeh, et al 1998 and Primo et al 2001).

Antioxidant and free radical scavenging activity of defatted and fractionated methanolic extract of aerial parts of *Lippia nodiflora* (MELN) was evaluated using invitro methods like 1, 1-diphenyl, 2-picryl hydrazine (DPPH) radical scavenging activity, H<sub>2</sub>O<sub>2</sub> scavenging activity, Nitric Oxide (NO) radical scavenging activity (Sangita Shukla, et al 2009). *Lippia nodiflora* has been reviewed for its biological activities and phytochemical constituents. antioxidant, antinociceptive, antimicrobial, antipyretic, antitumor, lipid peroxide scavenging and free radical scavenging activities. This review is aimed at collating all the scientific data available on the oil. (Faheem Amir, et al 2011 and Kunle Oluyemisi Folashade, et al 2012).

Medicinal plants contribute in human health care system. Most of the plants utilized by village people as a folk medicine. Now we are turned in to medicinal plant analysis of active compounds and conservation aspect. In the present study we had select the three important medicinal plants. We have collected two important medicinal plants such as *Lippia nodiflora* and *Vitex negundo* for antimicrobial studies. The experiment carried out in the selected medicinal plants leaves. (Malathi et al 2011).

*Vitex negundo* is an important medicinal plant with potent anti-inflammatory activity. The major constituents of this plant are flavonoids, casticin, chryso-splenol and vitexin, Chrysophenol D, nishindine and hydrocotylene anti-inflammatory, antioxidant, antinociceptive, anti-ulcer, free radical scavenging (Trapti Rastogi Meenal Kubde et al, 2010). Carrying out research to understand the genetic

mechanisms of the resistance in a better way and continuing investigations aimed at the development of new drugs from natural sources (Dash et al, 2001).

## **MATERIAL AND METHODS**

### **Plant collection and extraction**

The leaves of *Lippia nodiflora* (Linn.) and *Vitex negundo* (Linn.) were collected from in the around Namakkal district at June-2012. The fresh plants were collected in polythene bag and brought to the laboratories. First it washed with tap water, then surface sterilized in 10 per cent sodium hypochlorite solution to prevent the contamination of any microbes, then rinsed with sterile distilled water and air dried in shade at room temperature the samples were ground into a fine powder.

### **Preparation of plant extract**

Forty grams of the powdered leaves were loaded in Soxhlet apparatus and fractionated in 125 mL of (Methanol, chloroform, petroleum ether and hexane) solvent. The fraction was evaporated at rotary evaporator at 40C (Vogel 1978).

### **Method for fluorescence analysis**

Method for fluorescence analysis the method (Anonymous, 1999). Many phyto drugs when suitably illuminated emit light of different wave length or colour from that which falls on them. The fluorescence analysis of drug extract helps to identify the drug with specific fluorescent colours and to find out the fluorescent impurities. The study of fluorescence analysis can be used as a diagnostic tool for testing adulteration. The Powdered leaf parts were subjected to analysis under day/visible light and ultra violet light after treatment with various chemical as a part of Fluorescence analysis.

Phytochemical activity of *Lippia nodiflora* (Linn.) and *Vitex negundo* (Linn.)

The different phytochemical analysis of this method of (Harborne 1998 and Bhandary et al, 2012).

### **Alkaloids.**

Five ml of the extract was added to 2 ml of HCl. To this acidic medium, 1 ml of Dragendroff's reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.

### **Anthraquinones.**

Five ml of the extract solution was hydrolysed with diluted Conc. H<sub>2</sub>SO<sub>4</sub> extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones.

### **Flavonoids.**

One ml of the extract, a few drops of dilute sodium hydroxide was added. An intense yellow colour was produced in the plant extract, which become colourless on addition of a few drops of dilute acid indicates the presence of flavonoids.

### **Saponins.**

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

### **Steroids and Terpenoids.**

To 1 ml of extract of drug, 1 ml of chloroform, 2–3 ml of acetic anhydride and 1 to 2 drops of concentrated sulfuric acid were added. (Dark green coloration of the solution indicates the presence of Steroids and dark pink or red coloration of the solution indicate the presence of terpenoids).

### **Tannins**

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

### **Carbohydrate.**

Benedict's test – Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

### **Proteins.**

Biuret Test – Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink color.

### **Test pathogens.**

The test bacterial pathogens namely *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus mirabilis* and *Bacillus subtilis* were collected in Poultech Agro Research Centre, Namakkal. Tamilnadu, India.

### **Maintenance of test pathogenic culture.**

Nutrient agar slants were used to maintain the test pathogenic culture were inoculated in the slant by streaking and were incubated at 37°C for 24 hours and then stored at 4°C for further analysis.

### **Antibacterial activity.**

In the preliminary screening, the effect of different crude extracts of *Lippia nodiflora* (Linn.) and *Vitex negundo* (Linn.) leaf on bacterial growth was determined by agar well diffusion method (Bauer et al, 1966).

### **Minimum inhibitory concentration**

The microbroth dilution test was performed to (Jorgensen et al, 1999). Determine minimum inhibitory concentrations (MICs) 100 µl of Mueller-Hinton broth (MHB) were added into each well of a microtiter plate. The 100 µl aliquot of stock solution of crude extract (200 mg/ml in 10% DMSO) was added and subsequently 2-fold serially

diluted with broth.

#### Antioxidant assay.

DPPH Radical scavenging activity of *Lippia nodiflora* (Linn.) and *Vitex negundo* (Linn.).

DPPH radical-scavenging activity was determined by the method of (Shimada et al 1992). Extract stock solutions (1.0 mg/ml) were diluted to final concentrations of 10, 20, 30, 40 and 50 µg/ml in methanol. 1 ml of a 0.3 mM DPPH methanol solution was added to 2.5 ml solution of the extract or standard and allowed to react at room temperature for 30 min. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA%) using the formula:

AA% = [(Abs control - Abs sample) × 100] / Abs control  
Methanol (1.0 ml) plus extract solution (2.5 ml) was used as a blank. 1 ml of 0.3 mM DPPH plus methanol (2.5 ml) was used as a negative control.

#### Hydrogen peroxide scavenging activity assay

Hydrogen peroxide scavenging activity of the oleonic acid was estimated by replacement titration (Zhang 2000). Prepared in phosphate buffer (pH 7.4). Different concentrations (10, 20, 30, 40 and 50 µg/ml) of both extracts were added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min. against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of extract and standard compounds ascorbic acid was calculated using the following formula:

% scavenged [H<sub>2</sub>O<sub>2</sub>] = [(A<sub>0</sub>-A<sub>1</sub>)/A<sub>0</sub>] × 100 where A<sub>0</sub> was the absorbance of the control, and A<sub>1</sub> was the absorbance in the presence of the sample and standards.

## RESULTS

### Florescence analysis.

The florescence analysis of leaves powder of *Lippia nodiflora* (Linn.) and *Vitex negundo* (Linn.) The leaf powders are treated with various chemicals exhibited various colours in the visible and UV light. The leaf powders have light green and dark green in colour under visible light and short UV light were presented in table-1 and 2 respectively.

### Phytochemical analysis.

The phytochemical activities of different extract of (Methanol, Chloroform, Hexane, Petroleum ether) *Lippia nodiflora* (Linn.) *Vitex negundo* (Linn.) were presented in table-3 and 4 respectively.

### Antibacterial activity

Antibacterial activity of different leaves extract of *Lippia nodiflora* (Linn.) were tabulated in table-5. Among 4 different extract, methanol extract only more effective against *Salmonella typhi*, *Proteus mirabilis* and *Staphylococcus aureus* and *Bacillus cereus* with zone of inhibition 14, 12 and 10 mm respectively.

Antibacterial activity of different leaves extract of *Vitex negundo* were tabulated in table-6. Methanol extract

was the most effective against all bacterial pathogens. Fourteen mm zone of inhibition observed against *Pseudomonas aeruginosa* and *Salmonella typhi*, 13 mm against *Bacillus cereus* and *Escherichia coli*, 12 mm against *Staphylococcus aureus* and *Proteus mirabilis*. Chloroform extract showed the highest inhibition zone against *Salmonella typhi* while Hexane extract active against only *Bacillus cereus*.

Minimum inhibitory concentration of methanolic extract of *Lippia nodiflora* (Linn.) and *Vitex negundo* (Linn.)

The minimum inhibitory concentration of methanolic extract of *Lippia nodiflora* (Linn.) was presented in table-7. The extract showed 25mg/ml for *Salmonella typhi* and *Bacillus cereus*, 12.5mg/ml for *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, 6.25mg/ml for *Proteus mirabilis*.

The minimum inhibitory concentration of methanolic extract of *Vitex negundo* (Linn.) was presented in table-8. The MIC of methanol extract was 25 mg/ml for *Pseudomonas aeruginosa* and *Salmonella typhi*, 50mg/ml for *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Proteus mirabilis*.

### Antioxidant

DPPH radical scavenging activity of methanolic leaves extract of *Lippia nodiflora* (Linn.) and *Vitex negundo* (Linn.)

The DPPH radical scavenging activity of the methanolic leaves extract of *Lippia nodiflora* (Linn.) were tabulated in table-9. The DPPH activity of methanolic extract was 9.33, 13.06, 17.56, 25.47 and 31.50 % of inhibition in 10, 20, 30, 40 and 50 µg/ml of extract respectively. The standard as ascorbic acid was 25.13, 46.66, 64.43, 76.62 and 83.09 % of inhibition in 10, 20, 30, 40 and 50 µg/ml of extract respectively. The IC<sub>50</sub> value of methanolic extract and ascorbic acid were 79.37 and 30.09 µg/ml respectively.

The DPPH radical scavenging activity of the methanolic leaves extract of *Vitex negundo* were tabulated in table 10. The DPPH activity of methanolic extract was 8.87, 10.98, 13.39, 17.23 and 21.84 % of inhibition in 10, 20, 30, 40 and 50 µg/ml of extract respectively. The standard as ascorbic acid was 25.13, 46.66, 64.43, 76.62 and 83.09 % of inhibition in 10, 20, 30, 40 and 50 µg/ml of solution respectively. The IC<sub>50</sub> value of methanolic extract and ascorbic acid were 114.47 and 30.09 µg/ml respectively.

Hydrogen peroxide scavenging activity of methanolic leaves extract of *Lippia nodiflora* (Linn.) and *Vitex negundo* (Linn.)

Hydrogen peroxide scavenging activity of methanolic leaves extract of *Lippia nodiflora* (Linn.) were presented in table- 11. At 10, 20, 30, 40 and 50 µg/ml of extract of extracts were 0.06, 10.34, 14.10, 17.95 and 25.38 % inhibition of hydrogen peroxide scavenging activity, while ascorbic acid 40.94, 56.79, 60.04, 68.86 and 87.02 % of inhibition in 10, 20, 30, 40 and 50 µg/ml of solution respectively. The IC<sub>50</sub> value of methanolic extract and ascorbic acid were 97.05 and 28.73 µg/ml respectively.

Hydrogen peroxide scavenging activity of methanolic leaves extract of *Vitex negundo* (Linn.) were

presented in table- 12. At 10, 20, 30, 40 and 50µg/ml of extract of extracts were 17.04, 23.83, 30.12, 43.81 and 58.21 % inhibition of hydrogen peroxide scavenging activity, while ascorbic acid 40.94, 56.79, 60.04, 68.86 and 87.02 % of inhibition in 10, 20, 30, 40 and 50 µg/ml of solution respectively. The IC50 value of methanolic extract and ascorbic acid were 42.95 and 28.73 µg/ml respectively.

**Table-1: Fluorescence analysis of leaves powder of Lippia nodiflora**

S.No	Sample	White light	White UV	BlueUV
1	Powder	Light green	Dark green	Black
2	Powder+Water	Light yellow	Green	Black
3	Powder+Methanol	Green	Dark green	Black
4	Powder+Chloroform	Green	Dark Brown	Black
5	Powder+Petroleum ether	Green	Dark green	Black
6	Powder+Hexane	Green	Dark green	Black
7	Powder+Ethanol	Green	Green	Black
8	Powder+Ethyl acetate	Green	Dark green	Black
9	Powder+Acetone	Yellowish green	Light green	Black
10	Powder+Ammonia solution	Yellowish green	Green	Black

**Table-2: Fluorescence analysis of leaves powder of Vitex negundo**

S.No	Sample	White light	White UV	BlueUV
1	Powder	Light green	Dark green	Black
2	Powder+Water	Dark green	Dark green	Black
3	Powder+Methanol	Green	Dark green	Black
4	Powder+Chloroform	Green	Dark green	Black
5	Powder+Petroleum ether	Green	Dark green	Black
6	Powder+Hexane	Green	Dark green	Black
7	Powder+Ethanol	Green	Dark green	Black
8	Powder+Ethyl acetate	Green	Dark green	Black
9	Powder+Acetone	Dark green	Dark green	Black
10	Powder+Ammonia solution	Dark green	Dark green	Black

**Table-3: Photochemical activity of different solvent leaves extract of Lippia nodiflora**

S.No	Phytochemical	Methanol	Chloroform	Hexane	Petroleum ether
1	Alkaloids	+	+	+	-
2	Anthraquinones	+	-	-	-
3	Flavonoids	+	+	-	+
4	Saponins	+	+	+	+
5	Steroids	+	+	-	+
6	Terpenoids	-	-	-	-
7	Tannins	+	-	+	-
8	Carbohydrate	+	+	+	-
9	Proteins	-	-	-	-

(+) = Positive, (-) = Negative

**Table-4: Photochemical activity of different solvent leaves extract of Vitex negundo**

S.No	Phytochemical	Methanol	Chloroform	Hexane	Petroleum ether
1	Alkaloids	+	+	+	+
2	Anthraquinones	-	-	-	-
3	Flavonoids	+	-	-	-
4	Saponins	+	-	+	-
5	Steroids	-	+	-	+
6	Terpenoids	-	-	-	-
7	Tannins	-	-	+	-
8	Carbohydrate	-	-	-	-
9	Proteins	-	-	-	-

(+) = Positive, (-) = Negative

**Table-5: Antibacterial activity of different leaves extract of Lippia nodiflora (linn.)**

S. No	Bacterial strains	Zone of inhibition (diameter in mm)				
		Methanol	Chloroform	Hexane	Petroleum ether	Tetracycline
		10mg/well	10mg/well	10mg/well	10mg/well	30 µl of 10mg/ml
1	<i>Escherichia coli</i>	8	8	8	8	25
2	<i>Staphylococcus aureus</i>	10	8	8	9	29
3	<i>Pseudomonas aeruginosa</i>	9	8	8	8	27
4	<i>Salmonella typhi</i>	14	8	8	9	25
5	<i>Bacillus cereus</i>	10	9	8	8	22
6	<i>Proteus mirabilis</i>	12	9	8	8	23

**Table-6: Antibacterial activity of different leaves extract of Vitex negundo (Linn.)**

S. No	Bacterial strains	Zone of inhibition (diameter in mm)				
		Methanol	Chloroform	Hexane	Petroleum ether	Tetracycline
		10mg/well	10mg/well	10mg/well	10mg/well	30 µl of 10mg/ml
1	<i>Escherichia coli</i>	13	8	8	8	25
2	<i>Staphylococcus aureus</i>	12	8	8	8	29
3	<i>Pseudomonas aeruginosa</i>	14	8	9	8	27
4	<i>Salmonella typhi</i>	14	10	8	8	25
5	<i>Bacillus cereus</i>	13	9	10	8	22
6	<i>Proteus mirabilis</i>	12	8	8	8	23

**Table-7: Minimum inhibitory concentration of methanolic leaves extract of Lippia nodiflora (Linn.)**

Bacterial strains	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml	1.56 mg/ml	0.78 mg/ml	0.39 mg/ml	0.19 mg/ml	0.09 mg/ml	0.049 mg/ml
<i>Escherichia coli</i>	-	-	-	-	+	+	+	+	+	+	+	+
<i>Staphylococcus aureus</i>	-	-	-	-	+	+	+	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	-	-	-	-	+	+	+	+	+	+	+	+
<i>Salmonella typhi</i>	-	-	-	+	+	+	+	+	+	+	+	+
<i>Bacillus cereus</i>	-	-	-	+	+	+	+	+	+	+	+	+
<i>Proteus mirabilis</i>	-	-	-	-	+	+	+	+	+	+	+	+

(+) = Growth, (-) = No Growth

**Table-8: Minimum inhibitory concentration of methanolic leaves extract of Vitex negundo (Linn.)**

Bacterial strains	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml	1.56 mg/ml	0.78 mg/ml	0.39 mg/ml	0.19 mg/ml	0.09 mg/ml	0.049 mg/ml
<i>Escherichia coli</i>	-	-	+	+	+	+	+	+	+	+	+	+
<i>Staphylococcus aureus</i>	-	-	+	+	+	+	+	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	-	-	-	+	+	+	+	+	+	+	+	+
<i>Salmonella typhi</i>	-	-	-	+	+	+	+	+	+	+	+	+
<i>Bacillus cereus</i>	-	-	+	+	+	+	+	+	+	+	+	+
<i>Proteus mirabilis</i>	-	-	+	+	+	+	+	+	+	+	+	+

(+) = Growth, (-) = No Growth

**Table-9: DPPH radical scavenging activity of methanolic leaves extract of Lippia nodiflora (Linn.)**

S.No	Methanolic Concentration of extract	% of Inhibition	IC <sub>50</sub> µg/ml	Ascorbic acid	IC <sub>50</sub> µg/ml
1	10µg/ml	9.33	79.37	25.13	30.09
2	20µg/ml	13.06		46.659	
3	30µg/ml	17.56		64.43	
4	40µg/ml	25.47		76.72	
5	50 µg/ml	31.5		83.09	

**Table-10: DPPH radical scavenging activity of methanolic leaves extract of Vitex negundo (Linn.)**

S.No.	Methanolic Concentration of extract	% of Inhibition	IC <sub>50</sub> µg/ml	Ascorbic acid	IC <sub>50</sub> µg/ml
1	10µg/ml	8.87	114.47	25.13	30.09
2	20µg/ml	10.98		46.659	
3	30µg/ml	13.39		64.43	
4	40µg/ml	17.23		76.72	
5	50 µg/ml	21.84		83.09	

**Table-11: Hydrogen peroxide scavenging activity of methanolic leaves extract of Lippia nodiflora (Linn.)**

S.No.	Methanolic Concentration of extract	% of Inhibition	IC <sub>50</sub> µg/ml	Ascorbic acid	IC <sub>50</sub> µg/ml
1	10µg/ml	0.06	97.05	40.94	28.73
2	20µg/ml	10.34		56.79	
3	30µg/ml	14.10		60.04	
4	40µg/ml	17.95		68.86	
5	50 µg/ml	25.378		87.02	

**Table-12: Hydrogen peroxide scavenging activity of methanolic leaves extract of Vitex negundo (Linn.)**

S.No.	Methanolic Concentration of extract	% of Inhibition	IC <sub>50</sub> µg/ml	Ascorbic acid	IC <sub>50</sub> µg/ml
1	10 µg/ml	17.04	42.95	40.94	28.73
2	20 µg/ml	23.83		56.79	
3	30 µg/ml	30.12		60.04	
4	40 µg/ml	43.81		68.86	
5	50 µg/ml	58.21		87.02	

IC – Inhibitory concentration

## DISCUSSION

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action due to an alarming increase in the incidence of new and reemerging infectious diseases and development of resistance to the antibiotics in current clinical use. (Dimayuga et al 1991). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds (Edeoga et al, 2005). Many plant leaves have antimicrobial principles such as tannins, essential oils and other aromatic compounds. In addition, many biological activities and antibacterial effects have been reported for plant tannins and flavonoids [Kumar et al 1984 and Geissman 1963).

Preliminary phytochemical screening was helpful in prediction of nature of drugs and useful for the detection of different constituents present in different polarity solvent. So it could be helpful to extract out particular constituents by solvent (Jain et al 2011).

In antibacterial activity of different leaves extract of *Lippia nodiflora*, methanol extract only more effective against *Salmonella typhi*, *Proteus mirabilis* and *Staphylococcus aureus* and *Bacillus cereus* with zone of inhibition 14, 12 and 10 mm respectively. For *Vitex negundo* methanol extract was the most effective against all bacterial pathogens. Fourteen mm zone of inhibition observed against *Pseudomonas aeruginosa* and *Salmonella typhi*, 13 mm against *Bacillus cereus* and *Escherichia coli*, 12 mm against *Staphylococcus aureus* and *Proteus mirabilis*. In the present study, methanol extract is best solvent for antimicrobial activity of both selected plant against test pathogens. There are reports in the literature that methanol is a better solvent for consistent extraction of antimicrobial substances from medicinal plants. This is due to the stronger extraction capacity of methanol could have produced greater number/amount of active constituents responsible for antibacterial activity (Sengul et al 2005 and Ghosh et al 2008).

The minimum inhibitory concentration of methanolic extract of *Lippia nodiflora* (Linn.) showed 25mg/ml for *Salmonella typhi* and *Bacillus cereus*, 12.5mg/ml for *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, 6.25mg/ml for *Proteus mirabilis*.

The minimum inhibitory concentration of methanolic extract of *Vitex negundo* (Linn.) was 25 mg/ml for *Pseudomonas aeruginosa* and *Salmonella typhi*, 50mg/ml for *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Proteus mirabilis*.

In DPPH assay, the IC<sub>50</sub> value of methanolic extract of *L. nodiflora* and ascorbic acid were 79.37 and 30.09 µg/ml respectively. In DPPH assay, the IC<sub>50</sub> value of methanolic extract of *V. negundo* (Linn.) and ascorbic acid were 114.47 and 30.09 µg/ml respectively.

In H<sub>2</sub>O<sub>2</sub> scavenging activity, the IC<sub>50</sub> value of methanolic extract of *Lippia nodiflora* (Linn.) and ascorbic acid were 97.05 and 28.73 µg/ml respectively. In H<sub>2</sub>O<sub>2</sub> scavenging activity, the IC<sub>50</sub> value of methanolic extract of

*Vitex negundo* (Linn.) and ascorbic acid were 42.95 and 28.73 µg/ml respectively.

## CONCLUSION

Present investigations together with previous studies provide support to the rich phytochemical, antibacterial and antioxidant properties of *Lippia nodiflora* (Linn.) and *Vitex negundo* (Linn.). Therefore it can be used as antibacterial supplement in the developing countries towards the development of new therapeutic agents. Further pharmacological and clinical studies are required to understand the mechanism and the actual efficacy of these plant extracts in treating various infectious diseases. The phytochemical, antioxidant and antimicrobial activity medicinal plants showed significantly different due to different agro climatic environment and soil composition, geographical location and collection season.

## REFERENCE

1. Jeyachandran R, Baskaran X, Cindrella L (2010) Phytochemical and Antibacterial Potential of Four Indian Medicinal Plants. *J Med plants* 1(5): 301-306.
2. Rajasekara Pandiyan M, Sharmaila Banu G, Kumar G (2007) Antimicrobial activities of natural honey from medicinal plants on antibiotic resistant strains of bacteria. *Asian J Micro Biotec & Enviro Sci* 9: 219-224.
3. Bhagwati U (2003) Utilization of medicinal plants by the rural women of Kulu, Himachal Pradesh. *Indian J Tradit Know* 2: 366-270.
4. Dahanukar S A, Kulkarni A.R, Rege N N (2000) Pharmacology of medicinal plants and natural products. *Indian J Pharma* 32: 81-118.
5. Mahesh B, Sathish S (2008) Antimicrobial Activity of some important medicinal plant and Human pathogens. *World J Agri Sci* 4:839-843.
6. Cowan M.M (1999) Plant products as antimicrobial agents. *J Clin Microbio Review* 12: 564-582.
7. Nikaido H (1998) Multiple antibiotic resistance and efflux. *Cur Open Microbio* 1: 516-523.
8. Ali-Shtayeh M.S, Yagmour R.M.R, Faidi Y.R, Khalid, S, Al-Nuri, M.A (1998) Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. *J Ethnopharma* 60: 265-271.
9. Primo V, Rovera M, Zanon S, Oliva M, Demo V, Daghero J, Sabini L (2001) Determination of the antibacterial and antiviral activity of the essential oil from *Mintostachys verticillata* (Griseb.) Epling. *Revista Argen Microbio* 33: 113-117.
10. Sangita Shukla, Saluja A K, Pandya S S (2009) In vitro Antioxidant activity of Aerial parts of *Lippia nodiflora* Rich Pharmacologyonline. *J Pharm* 2: 450 – 459.
11. Faheem Amir, Wan Sinn Yam, Koay Yen Chin (2011) Chemical constituents and biological applications of *Lippia nodiflora*. *J Pharm* 2 (3): 101-105.
12. Kunle Oluyemisi Folashade, Egharevba Henry Omoregie (2012) Essential oil of *Lippia multiflora* Moldenke. *J Pharmace Sci* 1: 20-28.
13. Malathi R, Cholarajan A, Karpagam K, Jaya K R, Muthukumar P (2011) Antimicrobial studies on selected medicinal plants (*Coleus amboinicus*, *Phyllanthus nodiflora* and



- Vitex negundo). J Medic plant 5: 31-40.
14. Trapti Rastogi Meenal Kubde, Farooqui IA, Khadabadi S S (2010) A review on ethanomedicinal uses and phyto pharmacology of anti inflammatory herb Vitex negundo. J Ethnophar 1 (9): 23-28.
  15. Dash G.K, Suresh P, Ganapaty S (2001) Studies on hypoglycaemic and wound healing activities of Lantana camara Linn. J Nat Reme 1: 105-110.
  16. Vogel H (1978) Textbook of Practical Organic Chemistry. The English Language Book Society and Longman, London. pp. 1368.
  17. Anonymous (1999) The Ayurvedic Pharmacopoeia of India. Part I, Vol III, Government of India, Ministry of Health and Family Welfare. New Delhi. 235-238.
  18. Harborne J B (1998) Phytochemical methods. London Chapman and Hall, Ltd. pp: 49-188.
  19. Bhandary S K, Kumari N S, Bhat VS, Sharmila KP, Bekal M P (2012) Preliminary phytochemical screening of various extracts of Punica granatum peel, whole fruit and seeds. J Health Sci 2 (4): 35-38.
  20. Bauer AW, Kirby W M M, Sherris J C, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. American J Clini Patho 45: 493-496.
  21. Jorgensen JH, Turnidge JD, Washington JA (1999). Antibacterial susceptibility test: dilution and disk diffusion methods. Manual of Clinical Microbiology. Washington, D.C.: ASM Press pp. 1526-1562.
  22. Shimada K, Fujikawa K, Yahara K, Nakamura T (1992) Antioxidative properties of xanthum on the autoxidation of soybean oil in cyclodextrin emulsion. J Agri Food Chem 40: 945-948.
  23. Zhang X Y (2000) Principles of Chemical Analysis. Beijing: China Science Press 275-276.
  24. Dimayuga RE, Garcia SK (1991) Antimicrobial, screening of medicinal plants from Baja California sur, Mexico. J Ethnophar 31: 181-192.
  25. Edeoga , Okwu , Mbaebie (2005) Phytochemical constituents of some Nigerian medicinal plants. Afri. J Biotechnol. 4: 685-688.
  26. Kumar R, Singh M (1984) Tannins, their adverse role in ruminant nutrition. J Agric Food Chem 32: 447-453.
  27. Geissman TA (1963) Flavonoid compounds, tannins, lignins and related compounds. In: Florkin M and Stotz EH (eds). Pyrrole Pigments, Isoprenoid Compounds and Phenolic Plant Constituents, New York, USA: Elsevier Press, pp 265.
  28. Jain S, Sharma C, Khatri P, Jain A, Vaidya A (2011) Pharmacognostic and phytochemical investigations of the leaves of Zizyphus xylopyrus (Retz) wild, Int Pharm Sci, 3(2), 122-125.
  29. Sengul M, Ogutcu H, Adiguzel A (2005) Antimicrobial effects of Verbascum georgicum Bentham extract. Turk. J Biol, 29: 105-110.
  30. Ozturk S, Ercisli S (2006) The chemical composition of essential oil and in vitro antibacterial activities of essential oil and methanol extract of Ziziphora persica Bunge. J Ethnophar, 106: 372-376.
  31. Ghosh A, Das B K, Chatterjee S K Chandra G (2008) Antibacterial potentiality and phytochemical analysis of mature leaves of Polyalthia longifolia (Magnoliales:

Annonaceae). J Natural Sci, 26: 68-72.

# Publish Research Article International Level Multidisciplinary Research Journal For All Subjects

Dear Sir/Mam,

We invite unpublished research paper.Summary of Research Project,Theses,Books and Books Review of publication,you will be pleased to know that our journals are

## Associated and Indexed,India

- \* International Scientific Journal Consortium Scientific
- \* OPEN J-GATE

## Associated and Indexed,USA

- EBSCO
- Index Copernicus
- Publication Index
- Academic Journal Database
- Contemporary Research Index
- Academic Paper Databse
- Digital Journals Database
- Current Index to Scholarly Journals
- Elite Scientific Journal Archive
- Directory Of Academic Resources
- Scholar Journal Index
- Recent Science Index
- Scientific Resources Database

Golden Research Thoughts  
258/34 Raviwar Peth Solapur-413005,Maharashtra  
Contact-9595359435  
E-Mail-ayisrj@yahoo.in/ayisrj2011@gmail.com  
Website : www.isrj.net