PHARMACOLOGICAL ANALYSIS OF METHANOLIC LEAF EXTRACT OF *VITEX NEGUNDO* (LINN.) AND ITS INHIBITORY EFFECTS ON PATHOGEN CAUSING TYPHOID AND PNEUMONIA

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**KEYWORDS**

Life Extract  
Pneumonia  
Typhoid
In the present study, methanolic extracts of leaves of *Vitex negundo* L. was subjected to pharmacological analysis and was explored for possible anti-bacterial activity against *Salmonella typhi* and *Pseudomonas aeruginosa* by agar diffusion method. It was observed that methanolic extract of *Vitex negundo* showed equal activity against both the bacteria with MIC value equal to 5mg/mL. The leaf extracts was subjected to physiological, pharmacological tests and total ash value came to be 5.2%, Water soluble ash – 4%, Acid insoluble ash – 4%, Fibre content – 28.2%. The swelling index and foaming index came to be 100% and xx% respectively. Fat content was 7%, protein content was 13.785% and carbohydrate percentage was found to be 85%.

**ABSTRACT**

In the present study, methanolic extracts of leaves of *Vitex negundo* L. was subjected to pharmacological analysis and was explored for possible anti-bacterial activity against *Salmonella typhi* and *Pseudomonas aeruginosa* by agar diffusion method. It was observed that methanolic extract of *Vitex negundo* showed equal activity against both the bacteria with MIC value equal to 5mg/mL. The leaf extracts was subjected to physiological, pharmacological tests and total ash value came to be 5.2%, Water soluble ash – 4%, Acid insoluble ash – 4%, Fibre content – 28.2%. The swelling index and foaming index came to be 100% and xx% respectively. Fat content was 7%, protein content was 13.785% and carbohydrate percentage was found to be 85%.

**INTRODUCTION**

Traditional therapeutics based on herbal medicinal principles is time tested and widely accepted across various cultural and socio-economic strata. The systemic screening of plant species are performed in many laboratories (Davies, 1994). Typhoid fever is a global infection caused by the bacterium *Salmonella typhi*. The disease is transmitted by water, milk, fruits and vegetables contaminated with the bacterium. It is also transmitted by healthy carriers and contaminated food handlers. The bacilli may be carried mechanically from feces to food by flies. Reptiles such as snakes, turtles, lizards and common domestic pets have been associated with transmission of *Salmonella* spp (Birgitta et al., 2005). Typhoid fever can be treated with antibiotics. However, resistance to common antimicrobials is widespread. Healthy carriers should be excluded from handling food (WHO - http://www.who.int/topics/typhoid_fever/en/). In recent years there has been a rapid rise in multidrug resistance by *Salmonella typhi* all over the world (Chin et al., 2002; Benoit et al., 2003; Abdullah et al., 2005).

*Pseudomonas aeruginosa* is a common bacterium that can cause disease in animals including humans. It is found in soil, water, skin flora and most man-made environments throughout the world. It survives very well in hypoxic environments, and has thus colonized many natural as well as man-made environments. It is a Gram-negative, aerobic coccobacillus bacterium with unipolar motility (Ryan and Ray, 2004), an opportunistic pathogen for both plant and animals (Iglewski, 1996). *Pseudomonas aeruginosa* us capable of growing in diesel and jet fuel, where it is known as hydrocarbon using microorganism (HUM) bug, causing microbial corrosion (AVI Biopharma, 2007). In higher plants *Pseudomonas aeruginosa* induces symptoms of soft rot, as reported by Walker et al. in 2004 and Rahme et al., 1995; Rahme et al., 1997 in *Arabidopsis thaliana* and *Lactuca sativa* (lettuce). In the invertebrates it affects *Caenorhabditis elegans* (Mahajan et al., 1999; Martinez et al., 2004), *Drosophila* (D’Argenio et al., 2001), the moth *Calliaria mellonella* (Miya et al., 2003). In human beings it is an opportunistic, nosocomial pathogen of immunocompromised individuals, *Pseudomonas aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds and is also known to cause blood infections (Todar’s online textbook of Bacteriology, 2013). *Pseudomonas aeruginosa* in rare curcumstances cause community-acquired pneumonias (Fine et al., 1996) as well as ventilator-associated pneumonias, being one of the most common agents isolated in several studies (Diekema, 1999)

*Vitex negundo* Linn., belonging to family Verbenaceae (which comprises 75 genera and nearly 2500 species), commonly known as *Nirgundi* (Hindi), *Sindvar* (Sanskrit) and also called Five leaved chaste tree (Eng), is a deciduous shrub, occur in tropical to temperate regions (up to 2200 m from east to west) grows gregariously in wastelands and is also widely used as a hedge-plant. It is an erect (2–5 m in height), slender tree with quadrangular branchlets. The leaves have five leaflets in a palmately arrangement, which are lanceolate, 4–10 cm long, hairy beneath and pointed at both ends. The bluish purple flowers are numerous. The fruit is succulent, black and rounded when ripe having about 4 mm in diameter. *Vitex negundo* (Linn.) is
one of the common plants used in traditional medicine and reported to have variety of pharmacological activities. (Baral and Kurmi, 2006; Kirtikar and Basu, 1935; Kumar et al., 2013a). Although, all parts of *V. negundo* are used as medicine in the indigenous system of medicine, the leaves are the most potent for medicinal use. The decoction of leaves is used for treatment of inflammation, eye-disease, toothache, leucoderma, enlargement of the spleen, ulcers, cancers, catarhal fever, rheumatoid arthritis, gonorrhea, sinususes, scrofulous sores, bronchitis and as tonics, vermifuge, lactagogue, emmenagogue, antibacterial, antipyretic, antihistaminic, analgesic, insecticidal, ovicidal, feeding lactagogue, emmenagogue, antibacterial, antipyretic, antihistaminic, analgesic, insecticidal, ovicidal, feeding lactagogue, emmenagogue, antibacterial, antipyretic, antihistaminic, analgesic, insecticidal, ovicidal, feeding lactagogue, emmenagogue, antibacterial, antipyretic, antihistaminic, analgesic, insecticidal, ovicidal, feeding lactagogue, emmenagogue, antibacterial, antipyretic, antihistaminic, analgesic, insecticidal, ovicidal, feeding lactagogue, emmenagogue, antibacterial, antipyretic, antihistaminic, analgesic, insecticidal, ovicidal, feeding lactagogue, emmenagogue, antibacterial, antipyretic, antihistaminic, 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### MATERIALS AND METHODS

**Plant materials**
The leaves of *Vitex negundo* were collected in the month of February from Ranchi district of Jharkhand state. The leaf samples were shade dried on blotting paper for about 15 days for complete drying. They were then made to a fine powder by grinding in a mortar and pestle and stored at room temperature until use.

**Extraction**
The methanolic extracts of plant leaves were prepared by Soxhlet extraction method.

**Powder Preparation**
The plant leaves were washed with deionised water and disinfected with 0.1% HgCl2 solution for 5 min and dried in shade for 15 days. The dried materials were ground to fine powder with the help of electrical grinder (Jonani and Sondhi, 2002).

**Extract Preparation**
50 g of the sieved powder was weighed accurately and subjected to extraction in soxhlet apparatus using 350 ml methanol for preparation of methanolic extract. The obtained extract was concentrated with the help of vacuum rotatory evaporator at 45ºC. and stored.

**Physiological analysis**
The powdered plant leaves were analysed for physiochemical properties.

**Ash Content Analysis**
Total ash, Water soluble ash and Acid soluble ash was determined by following methods as per the protocols of – Quality control methods for medicinal plant materials, 1998.

**Determination of Crude Fibre**
The amount of crude fibre from the leaf extract of *Vitex negundo* was determined using the methods described by Watanable and Olsen, 1965.

**Determination of Moisture Content**
The moisture content of leaf extract of *Vitex negundo* was determined by using the methods described by Sadasivam and Manickam, 1996.

### Pharmacological Properties
The swelling index and foaming index were determined by following the methods described in – Quality control methods for medicinal plant materials, 1998.

### Nutritional Properties
The powdered sample was subjected to analysis for crude fat, proteins and carbohydrates as per previously described methods (Nile and Khobragade, 2009; Kumar et al., 2013a)

### Nutritive Value
The nutritive value was calculated on the basis of percentage of carbohydrate, protein and fat as per the formula used by Nile and Khobragade, 2009.

### Antibacterial analysis
The antibacterial analysis was performed by Agar diffusion and Broth dilution methods.

**Agar Diffusion Method**
Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 IL, 104 cfu) and spread evenly on the plate. After 20 min, the wells were filled with different concentrations of samples. The control wells were filled with Gentamycin along with solvent. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zones were noted (Threlfall et al., 1999; Walker, 2000; Kumar et al., 2013b)

**Media Used:** Media Used: Peptone-10 g, NaCl-10g and Yeast extract 5g, Agar 20g in 1000 mL of distilled water.

**Broth Dilution Method**
Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The tubes containing above media were prepared, autoclaved and respective concentrations of the samples were added. Each tube was inoculated with 18 h old cultures (100 IL, 104 cfu). A control tube with inoculums and without any sample was prepared along with a sterile media tube as blank. All the tubes were incubated at 37ºC on a shaker with 140 rpm for 24 h and the growth was measured at 660 nm (Threlfall et al., 1999; Walker, 2000).

**Media Used**
Peptone-10 g, NaCl-10g and Yeast extract 5g, in 1000 mL of distilled water

### RESULTS AND DISCUSSION

**Physiological Properties**
Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. Analytical techniques for providing information about the total mineral content are based

<table>
<thead>
<tr>
<th>SlNo.</th>
<th>Total ash</th>
<th>Water soluble ash</th>
<th>Acid insoluble ash</th>
<th>Crude fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.2</td>
<td>4</td>
<td>1.06</td>
<td>28.02</td>
</tr>
</tbody>
</table>
Table 2: percentage of Fat, Protein and Carbohydrate in *Vitex negundo*

<table>
<thead>
<tr>
<th>Sl no.</th>
<th>Crude fat</th>
<th>Crude protein</th>
<th>Crude carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>13.78.5</td>
<td>8.5</td>
</tr>
</tbody>
</table>

Table 3: MIC values obtained by broth dilution method

<table>
<thead>
<tr>
<th>Sl no.</th>
<th>Bacteria</th>
<th>MIC in mg/mL</th>
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</thead>
<tbody>
<tr>
<td></td>
<td><em>Vitex negundo</em></td>
<td>Gentamycin</td>
</tr>
<tr>
<td>1</td>
<td><em>Salmnella typhi</em></td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>5</td>
</tr>
</tbody>
</table>

Figure 1: Optical density at different concentrations obtained by broth dilution method

![Figure 1: Optical density at different concentrations obtained by broth dilution method](image1)

Figure 2: Percentage inhibition at different concentration obtained by broth dilution method

![Figure 2: Percentage inhibition at different concentration obtained by broth dilution method](image2)

Table 4: MIC values obtained by agar diffusion method in comparison with gentamycin

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Bacteria</th>
<th>0.13mg</th>
<th>0.36mg</th>
<th>0.612mg</th>
<th>1.25mg</th>
<th>2.5mg</th>
<th>5mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>S. typhi</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.45</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td><em>P. aeruginosa</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.99</td>
<td>4</td>
</tr>
</tbody>
</table>

Figure 3: A, B antibacterial analysis on *S. typhi* and *P. aerugiosa* (concentration in mg/mL) C, D. on *S. typhi* and *P. aerugiosa* Gentamycin (concentration in µg/mL) E: Methanolic leaf extracts of *Vitex negundo*

![Figure 3: A, B antibacterial analysis on *S. typhi* and *P. aerugiosa* (concentration in mg/mL) C, D. on *S. typhi* and *P. aerugiosa* Gentamycin (concentration in µg/mL) E: Methanolic leaf extracts of *Vitex negundo*](image3)

on the fact that the minerals can be distinguished from all the other components within a food in measurable way. The most widely used methods are based on the fact that minerals are not destroyed by heating, and that they have a low volatility compared to other food components.

The moisture content from the leaf extract of *Vitex negundo* was found to be 16.50 while the ash content from the leaf extract was found to be 59%. Ash content is important in some preparations (Ozcan et al., 2007). When leaf extract was incinerated, it left inorganic ash which in some cases varies within fairly wide limits. In many cases the total ash figure is within a characteristic narrow range and can be a useful characterization tool (Adikwu et al., 2001). The ash usually consists mainly of carbonates, phosphates, silicates and silica (Aziznia et al., 2008). This parameter gives an indication of the degree of mineral interaction in the structure and thus properties of the polysaccharides. *C. africana* had low ash content of 3.64 ± 0.01%. Ash content has been reported to be an important property and could be considered as purity parameter in leaf extracts. (Glicksman, 1969).

Dietary fibre is an imperative constituent of a balanced healthy diet. It has been reported that the increased incidences of degenerative diseases in the past century has been linked to the declining levels of fibre in the diet (Heller and Hackler, 1978; Trowell, 1976). *Vitex negundo* can be considered a rich source, the crude fiber content being 28.02 g%.

**Nutritional Properties**

The *Vitex negundo* powdered leaf sample was subjected to tests for investigating the percentage of fat, carbohydrate and protein. And it was observed that the sample is rich in protein. On comparing the results with that of various tropical and...
obtained by broth dilution method. and Fig. 2 show the values of optical density and % inhibition against both bacteria is shown in Table 6. Fig. 1 and table 5 respectively. The zone of inhibition obtained by agar diffusion against both bacteria is shown in Table 6. Fig. 1 and Fig. 2 show the values of optical density and % inhibition obtained by broth dilution method.

**REFERENCES**


Kumar, M., Dandapat, S., Kumar, A. and Sinha, M. P. 2013a Determination of Nutritive value and mineral elements of file-leaf chaste tree (Vitex neguno L.) and Malabar nut (Adhatoda vasica ). Academic J.plant sciences. 6(3): 103-108