In vitro antimicrobial activity and preliminary phytochemical screening of some plant extracts

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Abstract
Antimicrobial activity of five solvent extracts of local plants was evaluated in vitro, with four strains of bacteria viz., Salmonella typhi, Escherichia coli, Shigella flexneri (gram -ve), Staphylococcus aureus (gram +ve) and four strains of fungi viz., Alternaria alternata, Penicillium notatum, Aspergillus niger, Penicillium digitatum microorganism. The in vitro antibacterial and antifungal activities were tested by agar disc diffusion method. The most active antibacterial plants were Vitex negundo, Tagetes erecta and antifungal plants were Xanthium strumarium, Vitex negundo and Tagetes erecta, respectively. The significant antimicrobial activities of potent extracts were compared with the standard antimicrobics, Ciprofloxacin and Fluconazole for bacteria as well as fungi respectively at 1 mg/ml concentration. Preliminary phytochemical analysis of V. negundo leaf, X. strumarium, M. pruriens, C. bonduc seed and T. erecta flower extracts generally revealed the presence of Alkaloids, Steroids, Terpenoids, Phenols, Saponins, Anthraquinones, Amino acids, Carotenoids, Flavonoids and Tannins at various concentrations. The results obtained in this study suggest that X. strumarium, V. negundo, T. erecta can be used in treating diseases caused by these test organisms.

Keywords: Phytochemical, antimicrobial, plant extracts, Xanthium strumarium, Vitex negundo, Tagetes erecta, ciprofloxacin, fluconazole, etc.

Introduction
Bioactive compounds from vegetal sources are potential source of natural antifungic [1]. Many plants have great potential to produce new drugs which can be useful for human. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and even infectious diseases [2]. From ancient times, plants have provided tremendous support in traditional medicine systems as well as for the development of new potential drugs in modern pharmaceutical industries. Pathogenic bacteria have always been considered as a major cause of morbidity and mortality in humans. Though the pharmaceutical companies have produced a number of new antibacterials in the last few years, resistance to these drugs has increased and it has now become a global concern [3]. Due to the increase of resistance to antibiotics, the developments of new and innovative antimicrobial agents are utmost needed. Plants have long been investigated for the potential sources of new agents. Because, they contain many bioactive secondary metabolites that can be of interest in therapeutic [4]. To consider the immense importance of medicinal plants for therapeutic target, intensive studies have been performed on different plant extracts to isolate biologically active compounds [5]. Phytochemicals are non-nutritive plant chemical that have protective or diseases preventive properties. Plant produces these chemicals to protect itself, but recent research demonstrates that many phytochemicals in leaves and seeds works differently to protect humans against diseases [6].

Vitex negundo (Family: Verbenaceae) commonly known as Nirgudi, is an aromatic large shrub almost found throughout India is traditionally used for the treatment of skin- ulcers, as an insecticidal, antibacterial, antifungal, for rheumatoid arthritis, gonorrhea, bronchitis, inflammation, leucoderma, enlargement of the spleen, tumors and related diseases. Scientist from different areas has studied the significant biological activities of V. negundo plant and they evaluated its antibacterial [7-12], antifungal [8, 12-13] and cytotoxic activity [6].

Xanthium strumarium L. is an annual plant belonging to the family Asteraceae generally known as common cocklebur is available generally in between October and June in India. Various parts of this plant species were found to possess useful medicinal properties such as anthelmintic [14], antifungal [15], anti-ulcerogenic [16], and anti-inflammatory [17-18] activities; it is also known to inhibit proliferation of human cancer cells in vitro [19], [20] Srinivas et al., (2011),
reported high levels of bioactive compound group’s viz.,
alkaloids, phenolic acids and diterpenes and significant
concentrations of saponins, glycosides, fixed oils, and
phytosterols in \( \text{X. strumarium} \). \( \text{Tagetes erecta} \) commonly
known as marigold is a common ornamental herbaceous shrub
belonging to \( \text{Asteraceae} \) family with long history of
traditional medicinal use in many countries. It is used widely
in our Traditional System of Medicine for curing various
diseases \([20]\). Since ancient time parts of this plant has been
used for medicinal purposes and for the skin wash and yellow
dye used as by Cherokee \([21]\). Marigold is commonly used in
food additives as a coloring agent and as animal food in
fodder (dried flower meal and extract used as supplement for
poultry feed), also in tannin or dye industry \([22]\). It is also used
in medicine (folklore) and as a poison for non vertebrates and
in plant pest control \([23]\).

\( \text{Caesalpinia bonduc} \) is an Indian medicinal herb belonging to
\( \text{Caesalpiniaceae} \) family, found throughout India and other
tropical countries of the World \([25-26]\). \( \text{C. bonduc} \) seed are
traditionally used in the treatment of intermittent fever,
asthma, colic, antiperiodic, in dyspepsia, dentrifice and
filaria. Seed kernel is used in the treatment of orichitis,
ovariitis, scrofula, useful for dispersing swellings, restraining
hemorrhage in hydrocele leprosy and keeping off infectious
diseases \([27-29]\).

\( \text{Mucuna pruriens} \) (Family: \( \text{Fabaceae} \)), commonly called the
velvet bean is a popular Indian medicinal plant, which is used in
traditional Ayurvedic Indian medicine, for diseases
including Parkinsonism and widespread in tropical and sub-
tropical regions of the world. All parts of \( \text{M. pruriens} \) possess
valuable medicinal properties and it has been investigated in
various contexts, including for its anti-diabetic, aphrodisiac,
anti-neoplastic, anti-epileptic, and anti-microbial activities \([30]\).
This plant is widely used in Ayurveda, which is an ancient
traditional medical science that has been practiced in India
since the Vedic times 1500–1000 BC \([31]\).

**Material and Methods**

**Collection and Identification of Plant Material**

Fresh \( \text{Vitex negundo} \) leaves, \( \text{Xanthium strumarium} \) seeds,
\( \text{Caesalpinia bonduc} \) seeds, \( \text{Mucuna pruriens} \) seeds and
\( \text{Tagetes erecta} \) petals were collected from local, rural areas of
Aurangabad, Maharashtra, India. The taxonomic
identification of these plants was confirmed from Department
of Botany, Dr. Babasaheb Ambedkar Marathwada University,
Aurangabad. The plant material was washed under running
tap water, air dried for 2-3 weeks at room temperature (37 \(^\circ\)C
– 40 \(^\circ\)C) and pulverized in an electric grinder and stored in
airtight bottles.

**Preparation of the Extract**

20 gm of plant powder was soaked in 200 ml ethanol,
methanol, chloroform, acetone, n-Hexane solvents separately
and extracts were extracted with Soxhlet apparatus.

**Preparation of Disc**

Antimicrobial activity was planned to test using disc method.
For the disc preparation Whatman filter paper No.1 was used.
Whatman filter paper is folded and made a single layer. Later
with the help of punching machine, it was punched into small
pieces. These disc pieces were collected in the baby jar and
autoclaved and preserved for further research purpose.

**Observations and Results**

The extracts of \( \text{V. negundo} \) leaves in all five solvents showed
their strong activity against \( \text{E. coli} \). Subsequently, the extract
of \( \text{V. negundo} \) leaves in three solvents, \( \text{M. pruriens} \) seeds in
two solvents, \( \text{T. erecta} \) petals in three solvents showed strong
activity against \( \text{S. typhi} \) as compared to different solvent
extracts of \( \text{X. strumarium} \) and \( \text{C. bonduc} \) seeds. The different
solvent extracts of \( \text{T. erecta} \) petals, \( \text{C. bonduc} \) seeds and \( \text{V. negundo} \) leaves showed minimum activity against \( \text{S. flexneri} \)
and there is no effect of \( \text{M. pruriens} \) and \( \text{X. strumarium} \) seeds
on it. The extracts of \( \text{V. negundo} \) leaves, \( \text{T. erecta} \) petals showed highest activity against bacterial strain \( \text{S. aureus} \) and
other three plant species showed very less or negligible
activity against it.

**Preliminary Phytochemical Screening**

The major secondary metabolites like alkaloids, flavonoids,
saponins, phenols, terpenoids, antheraquione, proteins and
amino acids, carbohydrates and glycosides etc. were assessed
according to the standard procedure described by Harborne,
(1998) \([32]\).

**Antibacterial Screening**

**Test organisms**: A panel of bacterial strains including
\( \text{Escherichia coli, Salmonella typhi, Shigella flexneri} \) (gram -ve)
and \( \text{Staphylococcus aureus} \) (gram +ve) were used for
antibacterial activity. Standard strains were obtained from
NCL (National Chemical Laboratory), Pune, India. The
microorganisms were maintained at 4 \(^\circ\)C on Mueller Hinton
Agar (HIMEDIA) slants.

**Antifungal Screening**

**Test organisms**: A panel of fungal species including
\( \text{Alternaria alternata, Penicillium notatum, Aspergillus niger,}
\text{Penicillium digitatum} \) were used for antifungal activity.
Standard strains obtained from Mycology laboratory of
Department of Botany, Dr. Babasaheb Ambedkar
Marathwada University, Aurangabad (MS), India. The
microorganisms were maintained at 4 \(^\circ\)C on Sabouraud
Dextrose Agar (HIMEDIA) plates.

**Preparation of the Test Solutions and Antimicrobial Assay**

**Using Agar Disc Diffusion Methods**

Antimicrobial activity was carried out according to the
method of Bauer et al., (1966) \([33]\), Stock cultures were
maintained at 4 \(^\circ\)C on slope of Mueller Hinton Agar for
bacteria and plates of Sabouraud Dextrose Agar for fungi. 200
\( \mu \)l of standardized cell suspensions were spread on a Mueller
Hinton Agar. Extracts of all five plants (leaves and seeds)
dissolved in respective solvents and aliquot 10 \( \mu \)l of
extracts. The samples were applied to disc paper and air dried.
The air dried discs, with extract were inoculated on agar plate
against selected microorganisms. After 24 hrs of incubation
the results were recorded in different time interval. The
effects of plant extracts were compared with Ciprofloxicin and
Flucanazole as a standard for bacteria as well as fungi
respectively at 1 mg/ml concentration. The results obtained by
measuring diameters of the zone of inhibition in millimeter
(mm) are represented as, +++ = Maximum antibacterial/antifungal activity. ++ = Average antibacterial/antifungal activity and + = Minimum antibacterial/antifungal activity. The data obtained was tabularized and the necessary statistical applications were made.
The antifungal potential of *V. negundo* leaves, *X. strumarium* seeds and *T. erecta* petals was very strong against *A. alternata*. Subsequently, all the five plant extracts in different solvents showed their highest antifungal activity against *P. notatum*. The extracts of *V. negundo* leaves, *C. bonduc* seeds shows very less antifungal activity against *A. niger* and there is no any effect of *X. strumarium*, *M. pruriens* seeds and *T. erecta* petals in all solvent extracts against it. The antifungal activity of different solvent extracts of screened plants such as *X. strumarium*, *C. bonduc* seeds, *T. erecta* petals shows minimum activity as compared to *V. negundo* leaves against *P. digitatum*. There is no any effect of *M. pruriens* seed extracts in all the five solvents against *P. digitatum*.

Table No. 2 summarize the microbial growth of different solvent extracts of screened plants against gram –ve bacteria viz., *E. coli*, *S. typhi*, *S. flexneri* and gram +ve bacteria such as *Staphylococcus aureus* respectively. Table No. 3 summarize the fungicidal effect of different solvent extracts of screened plants.

There are many differences in the antibacterial as we as antifungal effects of plant species, due to the phytochemical properties and differences among microbes. It may quite possible that, some of the plants were ineffective in the present study do not possess antibiotic properties or the plant extracts may have contained antibacterial and antifungal constituents insufficient concentrations. The drying process may have caused conformational changes to occur in some of the chemical constituents found in these plants species. The secondary metabolites like tannins, flavonoids, steroids, saponins, alkaloids phenols, terpenoids, anthraquinone, proteins and amino acids present in trace amount in some of the plants are showed in Table No. 1.

**Discussion**

Each of the extract tested in the present study displayed antibacterial as well as antifungal activity on at least 2-4 bacterial and 2-4 fungal strains tested. However, differences were observed between antibacterial and antifungal activities of the extracts. These differences may be due to change in the chemical composition of these extracts as the bioactive compounds from plants have many effects including antibacterial and antiviral properties\[34-35\].

**Table 1:** Preliminary qualitative phytochemical analysis of five screened plants species

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Plant species</th>
<th>Plant part</th>
<th>Tannins</th>
<th>Saponins</th>
<th>Phenols</th>
<th>Flavonoids</th>
<th>Alkaloids</th>
<th>Steroids</th>
<th>Terpenoids</th>
<th>Anthraquinone</th>
<th>Amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Vitex negundo</em></td>
<td>Leaves</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td><em>Xanthium strumarium</em></td>
<td>Seed</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td><em>Caesalpinia bonduc</em></td>
<td>Seed</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td><em>Mucuna pruriens</em></td>
<td>Seed</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>5</td>
<td><em>Tagetes erecta</em></td>
<td>Petals</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

**Table 2:** Antibacterial activity of different solvent extracts of screened plants against Gram –ve and Gram +ve bacteria.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Plant Species</th>
<th>Plant Part</th>
<th>Bacterium</th>
<th>Control (Ciprofloxacin)</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Chloroform</th>
<th>Acetone</th>
<th>Hexane</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Vitex negundo</em></td>
<td>Leaves</td>
<td><em>E. coli</em> (Gram -ve)</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>S. typhi</em> (Gram -ve)</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>S. flexneri</em> (Gram -ve)</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>S. aureus</em> (Gram +ve)</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td><em>Xanthium strumarium</em></td>
<td>Seed</td>
<td><em>E. coli</em> (Gram -ve)</td>
<td>+++</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><em>S. typhi</em> (Gram -ve)</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>+</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><em>S. flexneri</em> (Gram -ve)</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><em>S. aureus</em> (Gram +ve)</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td><em>Caesalpinia bonduc</em></td>
<td>Seed</td>
<td><em>E. coli</em> (Gram -ve)</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>S. typhi</em> (Gram -ve)</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><em>S. flexneri</em> (Gram -ve)</td>
<td>+++</td>
<td>+</td>
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<td>+</td>
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<td>-</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><em>S. aureus</em> (Gram +ve)</td>
<td>+++</td>
<td>+</td>
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</table>

Rose and Cathrine (2011)\[36\], reported *E. coli* and *Streptococcus mutans* were resistant to three different solvent extracts of *V. negundo* leaves viz., petroleum ether, dichloromethane and ethanol. They also reported that *Klebsiella pneumoniae*, *Vibrio cholerae*, *Streptococcus mutans*, *Eschericia coli* have inhibitory activity next only to the *Salmonella paratyphi*. Lölge et al., (2016)\[37\], in three *C. auriculata*, *A. mexicana*, *S. trifolius* plant extracts in four different solvents; acetone, methanol, ethanol and chloroform showed their antibacterial activity against *E. coli* and *S. aureus*. Islam et al., (2013)\[38\], studied; Leaf extract of *V. negundo* in methanol at 10 mg/ml exhibited a variable growth inhibition capacity against all bacterial species viz., *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi*. Devkota and Das (2015)\[39\], showed the methanolic extract of *X. strumarium* shows zone of inhibition with *K. pneumoniae*, *E. faecalis*, *B. subtilis*, *P. mirabilis*, *S. aureus* but it was ineffective against *E. coli*. Similarly, distilled water extract of *X. strumarium* showed zone of inhibition with *E. faecalis*, *B. subtilis*, *P. mirabilis*, *S. aureus* and it was incapable with *E. coli* and *K. pneumoniae*. The leaf extract of *X. strumarium* in aqueous, ethanol and n-hexane solvents, inhibited *S. aureus* and not *E. coli*, *K. pneumoniae* and *B. subtilis*\[40\], but in the present study only n-Hexane extract of *X. strumarium* showed antibacterial effects against *S. aureus*. The extract of chloroform of *X. strumarium* inhibited *S. aureus*, *B. subtilis*, *E. coli* and ethyl acetate extract inhibited *S. aureus*\[41\], but no inhibition of any bacterial growth was observed by aqueous extract however we haven’t found any activity of chloroform extract of *X. strumarium* seed against *S. aureus* and *E. coli*. These differences in result may be due to the difference in time of collection of plant material, difference of solvent, dose and habitat of plant material. Verma and Verma (2012)\[21\], studied the antibacterial potential of ethanolic extracts from leaves, flower, stem and root of plant *T. erecta* and reported that *S. lutea*, *B. circulence*, *B. subtilis* were most sensitive to the leaf extract while *E. coli* and *S. aureus* were least sensitive to the ethanolic extract. Flower extract showed significant antibacterial activity against *S. lutea*, *E. coli*, *B. circulence.*
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