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# *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 antimicrobiotics, 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 *X. strumarium*. *Tagetes erecta* commonly known as marigold is a common ornamental herbaceous shrub belonging to 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 [21]. 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 [22]. 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 [23]. It is also used in medicine (folklore) and as a poison for non vertebrates and in plant pest control [24].

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

*Mucuna pruriens* (Family: 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 *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 *Vitex negundo* leaves, *Xanthium strumarium* seeds, *Caesalpinia bonduc* seeds, *Mucuna pruriens* seeds and *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 $^0$C – 40 $^0$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.

### Preliminary Phytochemical Screening

The major secondary metabolites like alkaloids, flavonoids, saponins, phenols, terpenoids, anthraquinone, 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 *Escherichia coli, Salmonella typhi, Shigella flexneri* (gram -ve) and *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 $^0$C on Mueller Hinton Agar (HIMEDIA) slants.

### Antifungal Screening

**Test organisms:** A panel of fungal species including *Alternaria alternata, Penicillium notatum, Aspergillus niger, 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 $^0$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 $^0$C on slope of Mueller Hinton Agar for bacteria and plates of Sabouraud Dextrose Agar for fungi. 200 µl of standardized cell suspensions were spread on a Mueller Hinton Agar. Extracts of all five plants (leaves and seeds) were dissolved in respective solvents and aliquot 10 µl 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 Ciprofloxacin and Fluconazole 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.

## Observations and Results

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

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].

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 *Klebsiela pneumoniae, Vibrio cholerae, Streptococcus mutans, Eschericia coli* have inhibitory activity next only to the *Salmonella paratyphi*. Lolge *et al.*, (2016) [37], in three *C. auriculata, A. mexicana, S. trifoliatus* 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. pneumonia*. 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*.

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

| Sr. No. | Plant species | Plant part | Tannins | Saponins | Phenols | Flavonoids | Alkaloids | Steroids | Terpenoids | Anthraquinone | Amino acids |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Vitex negundo* | Leaves | + | + | + | + | + | + | + | + | + |
| 2 | *Xanthium strumarium* | Seed | + | + | + | + | + | - | + | - | + |
| 3 | *Caesalpinia bonduc* | Seed | + | + | - | + | - | + | + | - | + |
| 4 | *Mucuna pruriens* | Seed | + | + | + | - | + | + | + | - | + |
| 5 | *Tagetes erecta* | Petals | + | + | + | + | + | + | - | - | + |

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

| Sr. No. | Plant Species | Plant Part | Bacterium | Control (Ciprofloxacin) | Ethanol | Methanol | Chloroform | Acetone | n-Hexane |
|---|---|---|---|---|---|---|---|---|---|
| 1. | *Vitex negundo* | Leaves | *E. coli* (Gram -ve) | +++ | +++ | +++ | ++ | +++ | + |
| | | | *S. typhi* (Gram -ve) | +++ | ++ | ++ | + | ++ | - |
| | | | *S. flexneri* (Gram -ve) | +++ | ++ | ++ | - | ++ | ++ |
| | | | *S. aureus* (Gram +ve) | +++ | +++ | +++ | ++ | +++ | - |
| 2. | *Xanthium strumarium* | Seed | *E. coli* (Gram -ve) | +++ | - | - | - | - | - |
| | | | *S. typhi* (Gram -ve) | +++ | - | + | - | + | + |
| | | | *S. flexneri* (Gram -ve) | +++ | - | - | - | - | - |
| | | | *S. aureus* (Gram +ve) | +++ | - | - | - | - | + |
| 3. | *Caesalpinia bonduc* | Seed | *E. coli* (Gram -ve) | +++ | - | - | - | - | - |
| | | | *S. typhi* (Gram -ve) | +++ | + | + | ++ | + | - |
| | | | *S. flexneri* (Gram -ve) | +++ | + | + | ++ | + | - |
| | | | *S. aureus* (Gram +ve) | +++ | + | - | + | + | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 4. | *Mucuna pruriens* | Seed | *E. coli* (Gram -ve) | +++ | - | - | - | - | - |
| | | | *S. typhi* (Gram -ve) | +++ | + | + | ++ | ++ | - |
| | | | *S. flexneri* (Gram -ve) | +++ | - | - | - | - | - |
| | | | *S. aureus* (Gram +ve) | +++ | + | - | - | +++ | - |
| 5. | *Tagetes erecta* | Petals | *E. coli* (Gram -ve) | +++ | - | - | - | - | - |
| | | | *S. typhi* (Gram -ve) | +++ | +++ | ++ | + | +++ | - |
| | | | *S. flexneri* (Gram -ve) | +++ | ++ | ++ | ++ | + | + |
| | | | *S. aureus* (Gram +ve) | +++ | +++ | +++ | + | +++ | - |

**Table 3:** Antifungal activity of different solvent extracts of screened plants against four fungal strains.

| Sr. No. | Plant Species | Plant Part | Fungal Strain | Control (Fluconazole) | Ethanol | Methanol | Chloroform | Acetone | n-Hexane |
|---|---|---|---|---|---|---|---|---|---|
| 1. | *Vitex negundo* | Leaves | *Alternaria alternata* | +++ | ++ | ++ | ++ | + | - |
| | | | *Penicillium notatum* | +++ | ++ | + | +++ | ++ | - |
| | | | *Aspergillus niger* | +++ | ++ | + | + | + | + |
| | | | *Penicillium digitatum* | +++ | - | - | - | - | ++ |
| 2. | *Xanthium strumarium* | Seed | *Alternaria alternata* | +++ | + | ++ | +++ | + | ++ |
| | | | *Penicillium notatum* | +++ | + | + | ++ | + | ++ |
| | | | *Aspergillus niger* | +++ | - | - | - | - | - |
| | | | *Penicillium digitatum* | +++ | +++ | + | + | + | - |
| 3. | *Caesalpinia bonduc* | Seed | *Alternaria alternata* | +++ | - | - | ++ | ++ | - |
| | | | *Penicillium notatum* | +++ | ++ | + | + | - | ++ |
| | | | *Aspergillus niger* | +++ | - | - | +++ | + | + |
| | | | *Penicillium digitatum* | +++ | + | + | ++ | + | - |
| 4. | *Mucuna pruriens* | Seed | *Alternaria alternata* | +++ | - | - | - | - | - |
| | | | *Penicillium notatum* | +++ | + | + | ++ | ++ | + |
| | | | *Aspergillus niger* | +++ | - | - | - | - | - |
| | | | *Penicillium digitatum* | +++ | - | - | - | - | - |
| 5. | *Tagetes erecta* | Petals | *Alternaria alternata* | +++ | ++ | + | ++ | + | - |
| | | | *Penicillium notatum* | +++ | +++ | +++ | ++ | +++ | - |
| | | | *Aspergillus niger* | +++ | - | - | - | - | - |
| | | | *Penicillium digitatum* | +++ | ++ | + | + | - | + |

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