MATERIAL AND GENERAL METHODS
Collection of plants:

Seasonal surveys were made to collect plants from Marathwada. Plant materials were collected from the region and were identified after critical examination in the PG Department of Botany, Shivaji Mahavidyalaya, Udgir following standard flora (Naik.1998). Herbarium sheets were prepared and voucher specimens are deposited in the herbarium of PG Department of Botany, Shivaji Mahavidyalaya, Udgir.

Cultures:

The Cultures of the bacteria used *Escherichia coli* and *Staphylococcus aureus* were procured from Department of Microbiology, Shri Guru Gobind Singhji Memorial Hospital, Nanded. While culture of *Corynebacterium sp.* was provided by National Chemical Laboratory, Pune.

The bacterial cultures of *Xanthomonas malvacerum* was obtained from Indian Agricultural Research Institute, New Delhi.

The fungal human pathogen culture of *Candida albicans* and *Trichophyton rubrum* were obtained from Department of Microbiology, Government Medical College, Aurangabad.

The cultures of plant pathogens viz., *Alternaria solani* and *Helminthosporium turcicum* were obtained from Botany Research Laboratory, Science College, Nanded.

The cultures were maintained on the medium suggested by the respective laboratory and sub-culturing was done fortnightly. The cultures were incubated in an incubator, while the cultures were stored in refrigerator.
<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Organism</th>
<th>Culture Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Escherichia coli</em></td>
<td>Nutrient Agar</td>
</tr>
<tr>
<td>2</td>
<td><em>Staphylococcus aureus</em></td>
<td>Nutrient Agar</td>
</tr>
<tr>
<td>3</td>
<td><em>Corynebacterium sp.</em></td>
<td>Nutrient Agar</td>
</tr>
<tr>
<td>4</td>
<td><em>Xanthomonas malvacerum</em></td>
<td>Nutrient Agar</td>
</tr>
<tr>
<td>5</td>
<td><em>Candida albicans</em></td>
<td>Sabouraud Agar</td>
</tr>
<tr>
<td>6</td>
<td><em>Trichophyton rubrum</em></td>
<td>Sabouraud Agar</td>
</tr>
<tr>
<td>7</td>
<td><em>Alternaria solani</em></td>
<td>Malt Extract Agar</td>
</tr>
<tr>
<td>8</td>
<td><em>Helminthosporium turcicum</em></td>
<td>Malt Extract Agar</td>
</tr>
</tbody>
</table>

**Media:**

The following media were used in this study

1. **Sabouraud Agar**
   - Dextrose: 40gm
   - Peptone: 10gm
   - Agar: 20gm
   - Distilled water: 1000ml
   - pH: 5.5

2. **Malt extract agar (MEA)**
   - Malt extract: 20gm
   - Dextrose: 20gm
   - Peptone: 1gm
   - Agar: 20gm
   - Distilled water: 1000ml
   - pH: 5.5
3. **Nutrient Agar**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
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<tbody>
<tr>
<td>Beef extract</td>
<td>1gm</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>2gm</td>
</tr>
<tr>
<td>Peptone, bacteriological</td>
<td>5gm</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5gm</td>
</tr>
<tr>
<td>Agar</td>
<td>15gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
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</tbody>
</table>

The media were sterilized at pressure 15 lb. for 20 minutes in an Autoclave. 8ml of medium was poured in each test tube for slant preparation and 25ml of liquid media was poured in each 250ml conical flask for enzyme production.

**Culture Vessels:**

All the glass wares were thoroughly cleaned with acid-dichromate mixture and then washed with hot water and rinsed with distilled water and dried completely before use.

**Inoculum:**

Spore/cell suspension was prepared by adding sterile water to 8 day old for fungal and 48 hr old for bacterial MEA/SA/NA slant culture and 0.5ml of it was used as inoculum in all experiments. In every case spore suspension was standardized to contain $1 \times 10^5$ spore/ml for fungi and $1 \times 10^7$ cells/ml for bacteria by using haemocytometer. All the treatments/samplings were done in triplicates and results have been presented after repeating the experiment.
**Incubation:**

Cultures were incubated at 27 ± 2°C in the laboratory and as far as possible the temperature was maintained constant during the incubation and course of study. In all experiments the cultures were incubated for 8 days.

**Preparation of Powder:**

The plant materials collected were processed and used in this study within the year of collection. The plant parts collected were shredded and dried completely at 50°C for 72 h. The dried material were then ground into fine powder and stored in airtight containers at room temperature till extraction. Crude extracts were prepared from the same plants by extracting 2g dried material with 20ml distilled water, ethanol and ethyl acetate under for 30 min, respectively. Extracts were filtered and dried under vacuum. The samples were then air dried and redissolved to 10ml solution for antimicrobial testing.

**Extraction of Plant Tissue:**

The plant part powder was added to distilled water/ ethyl alcohol/ ethyl acetate and was allowed to boil for further 4-5 minutes on a water-bath under hood. 10ml of ethanol was used for every gram of powder. The extract was cooled; the powder was crushed thoroughly in a mortar and pestle and passed through several layers of muslin cloth. The ground powder was re-extracted by boiling in alcohol for 3min. to ensure the complete removal of contents. The extracts were pooled, centrifuged at 5000rpm and the volume was adjusted to represent 10 ml/gram of fresh weight of tissue (ml/gfw).
This extract was directly used for analysis qualitatively and quantitatively following methods suggested by Harborne (1984) and Mahadevan and Sridhar (1986).

**Plant Extract for Spore Germination**

The alcohol extract already estimated for Total phenols, was used to study the effect of phenols in the extract on spore germination.

5 ml of alcohol extract was evaporated on water bath under hood and slowly sterile distilled water was added to make up the volume to 5ml. This water extract was used to study the spore germination in host extract.

**Spore Germination:**

The spore suspension for germination studies was prepared by adding 5 ml of sterile distilled water to a heavily sporulating 8 days old slant culture of fungus. The suspension was filtered through several layers of muslin cloth to reduce the mycelial fragments. Spores were washed with sterile distilled water several times by centrifugation to remove nutrients from original medium. The spore finally obtained were diluted in water and adjusted to the desired concentration. The spore concentration was measured by Haemocytometer with Naubauer Counting Chamber (Deshpande and Papdiwal, 1979). Hanging drop slides were prepared for observation from the suspension and incubated in petriplates lined with moist blotters. Germination percentage was calculated by observing a minimum of 200 spores from different microscopic fields.

The spore germination in plant extract was compared with systemic fungicide Carbendazim. 1gm of technical grade Carbendazim obtained form
BASF, Mumbai was dissolved in 1000ml of sterile distilled water. This stock solution was used in spore germination studies.

**Plant Extract for Antibacterial and Antifungal Properties**

Antimicrobial activities of the plant extracts of the extract (free from alcohol/ethyl acetate and converted into aqueous) was evaluated by well-diffusion method expressed by zone of inhibition mm in diameter for *Candida albicans, Trichophyton rubrum*, and bacterial test organisms.

The bioassay was carried out by using 1ml of inoculum (1X10^6 colony forming units) prepared from an overnight Nutrient Broth culture or Sabouraud agar culture for given test bacteria or fungi were used. 1ml of the resultant spore/cell suspension was poured in the Petri plate and the plates were poured with respective medium was used to seed each prepared plate. The medium were allowed to solidify. Using a sterilized cork borer wells of 5mm diameter were made in the solidified inoculated medium and the plate area uniformly. The wells were filled with 0.5ml of extract. Plates were then incubated aerobically at 37°C for 24 h for bacteria and at 25°C for 72 h for fungi.

Similarly, wells containing standard concentration of antibiotics (Streptomycin and Tetracycline) were used to compare the antibacterial property of the plant extract. Pure antibiotics (Streptomycin and Tetracycline) were purchased from Hi-media, Mumbai. One hundred mg of each antibiotic was dissolved separately in 1000 ml sterile distilled water, 0.5ml was used to fill the wells. Amphotericin B was purchased from Hi-media, Mumbai and 1gm was dissolved separately in sterile distilled water, 0.5ml was used to fill the wells. These pure antibiotic solution were used for comparison.
Chromatographic Studies / Analysis

Two grams of dried powdered material was ground with 2ml 10% ammonia solution and then mixed with 7g basic aluminium oxide (activity grade I). This mixture was then packed loosely into a glass column and 10ml CHCl₃ was added. The alkaloids were eluted with 5ml CHCl₃. The elute was collected and evaporated down to 1ml and used for TLC. TLC studies were carried out employing Aluminium thin layer chromatography plates (silica gel 60 F 254; 20×20 cm) (Germany) and preparative thin layer chromatography glass plates (silica+indicator, 1mm, G 1510/LS 254; 20×20 cm) were purchased from Merck.

1 ml of alcohol extract of each sample was concentrated to 0.1ml and these samples were applied on one side of the TLC plate on precoated Silica gel G plates (E. Merck & Co., Damstadt, Germany) manufactured with fluorescent indicator using micropipette.

The plates were developed at room temperature using following solvent system.

n-butanol : Acetic acid : Water B A W (4:1:1) v/v

After development the TLC plates were observed under UV light before and after fuming plates with vapours of Ammonia (Hammerschmidt and Nicholson, 1977).

FUNGITOXICITY OF INDUCED PHENOLS

The TLC plates developed with treated and untreated samples were observed under UV light. The phenolic compound which had increased after treatment were marked /located in UV light and the silica gel containing these phenols were removed from the plates with razor blade.
Silica gel containing these phenols was placed in microcolumn constructed by plugging the open end of disposable tip of micropipette with glass wool. Phenols were eluted from Silica gel by passing 2ml of absolute alcohol through micro-column and were evaporated to dryness on water bath and 1ml of water was added.

This aqueous test solution of each spot was used for fungitoxicity assay by inhibition of germ tube length.

**Ethno-botanical information**

The compilation of the data is based on intensive field excursions during 1998-2003. The plant species collected during these field trips were identified. Ethno-botanical information presented here was gathered with the help of local informants and other elders of the various communities. The traditional medical practitioners were also consulted and were persuaded to accompany us on the field trips. Information was also collected from traditional healers, herbalists and rural dwellers of the villages of all the districts of Marathwada and adjoining areas. Through general interviews and by personal discussion, the information was collected about the names of plants used for the treatment, the parts of plants used, methods of preparation of plant materials, personal experience of users as well as their beliefs in the herbal treatment. Clinics and hospitals were visited and health personnel provided information regarding their knowledge of the local plants in the area.

The details of procedure used in addition have been specified at appropriate places.