INTRODUCTION

The medicinal plants are the plants whose parts (leaves, seeds, stem, roots, fruits, foliage etc.) extracts, infusions, decoctions, powders are used in the treatment of different diseases of humans. Medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth (antimicrobial activity). Higher plants have been shown to be a potential source for new anti-microbial agents. Today plant based drugs play as important role in traditional as well as conventional medicine throughout the world. The demand for herbal medicines continuously increased due to their lesser side effects when compared with synthetic drugs. Pharmaceutical companies largely depend upon materials procured from naturally occurring stands causing rapid depletion of this important source of medicinal herb. Hence, it has become imperative to establish a suitable protocol to generate enough materials to ensure its supply for pharmaceutical industries without further depleting this species. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world.

Oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems. Catalase and hydro peroxidase enzymes convert hydrogen peroxide and hydro peroxides to non radical forms and function as natural antioxidants in human body. Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions. In longer term, medicinal plants have identified as having high levels of antioxidant activity. Assaying in vitro may be of value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals-induced tissue damage. Solanum xanthocarpum (Solanaceae) is used as Carminative, Expectorant or decongestant.

Keywords: Ethernetic crude extracts, Terminalia chebula, Folk medicines, arthritis, phytopathogens, Enzymatic and non-enzymatic antioxidants

ABSTRACT

In vitro screening of ethanolic crude extracts of Indian medicinal plants Terminalia chebula, Centella asiatica, Solanum xanthocarpum were studied that have been popularly used as folk medicines. The main characteristic of an antioxidant is its ability to trap free radicals and also reduce the risk of chronic diseases including cancer, central nervous system injury, arthritis and heart diseases. Scientific information on antioxidant properties of various natural sources is still rather scarce. A variety of free radical scavenging antioxidants are found in plants. The results provided evidence that the studied plants might indeed be potential sources of natural antioxidant and antimicrobial agents.

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In vitro Bioautography of different Indian Medicinal plants

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It has been established that oxidative stress is among the major causative factors induction of many chronic and degenerative diseases and free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, is chemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS. Considerable scientific evidence suggested that under situations of oxidative stress reactive oxygen species (ROS) such as superoxide, hydroxyl and peroxyl radicals are generated and the balance between antioxidation and oxidation is believed to be a critical concept for maintaining a healthy biological system.

Terminalia chebula (Combretaceae) is used in India to treat many diseases such as digestive diseases, urinary diseases, diabetes, skin diseases, parasitic infections, heart diseases, irregular fevers, flatulence, constipation, ulcers, vomiting, colic pain and hemorrhoids. If taken after meals it prevents imbalance of any of the humors due to bad food or drink. It promotes wisdom, intellect and eyesight. It has a strong effect against the herpes simplex virus HSV, has antibacterial activity and exhibits strong cardio tonic properties. Terminalia

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Chebula also has an antioxidant component which indicates it can increase the life of tissues. Chebula is excellent for the digestive system.

_Centella asiatica_ (Mackinlayaceae) is a small herbaceous annual plant native to India, Sri Lanka, northern Australia, Indonesia, Iran, Malaysia, Melanesia, New Guinea, and other parts of Asia. The flowers are pinkish to red in color, born in small, rounded bunches (umbels) near the surface of the soil. Each flower is partly enclosed in two green bracts. The hermaphrodite flowers are minute in size (less than 3 mm), with 5-6 corolla lobes per flower. Each flower bears five stamens and two styles. The fruit are densely reticulate, distinguishing it from species of Hydrocotyle which have smooth, ribbed or warty fruit. The crop matures in three months and the whole plant, including the roots, is harvested manually. In Telugu Language this is known as “Saraswathi Plant” in India. _C. asiatica_ in an important medicinal plant in several ayurvedic preparations. It is reported to possess antipyloric, antileprotic, antibacterial and wound healing properties. The isolated steroids from the plant have been used to treat leprosy. It has anti-oxidant properties and re-vitalize the brain and nervous system, increase attention span and concentration. In thailand used for opium detoxification.

In this paper we report the results of such studies in order to orient future investigations towards the finding of potent and safe antimicrobial compounds and good source of natural antioxidants.

**MATERIALS AND METHODS**

**Solvents and chemicals used:**

All chemicals were purchased from Merck, Qualigens fine Chemicals and SD fine chemicals, Mumbai.

**Extraction procedure for antimicrobial:**

All medicinal plant material collected from various places of Andhra Pradesh they were taxonomically identified and the Voucher specimen is stored. The plant material were dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight container. The powder obtained was subjected to successive soxhlet extraction with organic solvents with increasing order of polarity i.e. Hexane, Chloroform and Methanol respectively.

**Extraction for procedure antioxidant activity:**

One hundred grams (100 g) of each plant material was shaken separately in methanol for 48 hrs on an orbital shaker. Extracts were filtered using a Whatman No 1 filter paper. Each filtrate was concentrated and each extract was resuspended in methanol to make 100 mg/ml stock solution.

**Test microorganisms:**

_Alternaria alternate_ (MTCC 1362), _Aspergillus flavus_ (MTCC 4633), _Aspergillius niger_ (MTCC 2723), _Bipolaris bicolor_ (MTCC 2105), _Cladosporium herbarum_ (MTCC2143), _Curvularia lunata_ (MTCC 2030), _Erwinia caratovora_ (MTCC 3609), _Fusarium oxysporum_ (MTCC 1755), _Macrophomina phaseolina_ (MTCC2165), _Pencillium expansum_ (MTCC 2006), _Pseudomonas syringae_ (MTCC 1604), _Pseudomaonas marginales_ (MTCC 2758), _Rhizoctonia solani_ (MTCC 4633), _Ustilago maydis_ (MTCC 1474), _Xanthomonos compstesies_ (MTCC 2286), including both fungi and bacteria were procured from Microbial Type Culture Collection (MTCC), Chandigarh. Active cultures were generated by inoculating a loopful of culture in separate 100mL nutrient/potato dextrose broth and incubating on a shaker at 37°C overnight. The cells were harvested by centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted in normal saline to obtain 5 x 10⁵ cfu/mL.

**Determination of antimicrobial activity:**

The crude extracts of the different plant parts of different species were subjected to antimicrobial assay using the agar well diffusion method of (Murray et al., 1995) modified by (Olurinola 1996) 20 ml of nutrient agar was dispensed into sterile universal bottles these were then inoculated with 0.2 ml of cultures mixed gently and poured into sterile petri dishes. After setting a number 3-cup borer (6mm) diameter was properly sterilized by flaming and used to make three to five uniform cups/wells in each petri dish. A drop of molten nutrient agar was used to seal the base of each cup. The cups/wells were filled with 50μl of the extract concentration of 500mg/ml, 250mg/ml and 100mg/ml and allow diffusing for 45 minutes. The solvents used for reconstituting the extracts were similarly analyzed. The plates were incubated at 37°C for 24 hours for bacteria. The above procedure is allowed for fungal assays but except the media potato dextrose agar instead of nutrient agar and incubates at 25°C for 48 hours. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in duplicates. The extracts and the phytochemicals that showed antimicrobial activity were later tested to determine the Minimal Inhibitory Concentration (MIC) for each bacterial and fungal sample.

**Minimum inhibitory concentration (MIC) assays:**

Based on the preliminary screening chloroform and methanolic extracts were found to have potent antimicrobial activity and Minimum Inhibitory Concentrations (MIC) of the extracts was determined according to Elizabeth. A final concentration of 0.5% (v/v) Tween-20 (Sigma) was used to enhance crude extract solubility. A series of two fold dilution of each extract, ranging from 0.2 to 100 mg/ml, was prepared. After sterilization, the medium was inoculated with 31 aliquots of culture containing approximately 10⁶ CFU/ml of each organism of 24 hours slant culture in aseptic condition and transferred into sterile 6 inch diameter petri dishes and allowed to set at room temperature for about 10 minutes and then kept in a refrigerator for 30 minutes.

After the media solidified a number 3-cup borer (6mm) diam-
Table 1

<table>
<thead>
<tr>
<th>Phytopathogens</th>
<th>Medicinal plants</th>
<th>SOD (U/mg)</th>
<th>Catalase (U/mg)</th>
<th>Ascorbic acid mg / 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. asatica</td>
<td>S. xanthocarpum</td>
<td>T. chebula</td>
<td></td>
</tr>
<tr>
<td>A. alternata</td>
<td>8</td>
<td>10</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>A. flavus</td>
<td>10</td>
<td>11</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>A. niger</td>
<td>-1</td>
<td>11</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>B. bicolor</td>
<td>11</td>
<td>12</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>C. luana</td>
<td>-</td>
<td>-</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>C. harbarum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>E. carotovora</td>
<td>16</td>
<td>13</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>F. oxyxporum</td>
<td>-</td>
<td>9</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>M. phaseolina</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>P. expansum</td>
<td>13</td>
<td>10</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>P. marginales</td>
<td>15</td>
<td>12</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>P. syringe</td>
<td>16</td>
<td>11</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>R. solani</td>
<td>10</td>
<td>11</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>U. maydis</td>
<td>12</td>
<td>11</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>X. campesiris</td>
<td>13</td>
<td>12</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

Methanolic extract concentration DMSO (100 mg/ml)

* All values indicates antimicrobial activity in mm
* (-) Value indicates no activity.

Table 2

<table>
<thead>
<tr>
<th>Name of the medicinal plants</th>
<th>Parts examined</th>
<th>SOD (U/mg)</th>
<th>Catalase (U/mg)</th>
<th>Ascorbic acid mg / 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. asatica</td>
<td>Flowers</td>
<td>1.04 ± 0.024</td>
<td>1.4 ± 0.06</td>
<td>120</td>
</tr>
<tr>
<td>S. xanthocarpum</td>
<td>leaves</td>
<td>3.9 ± 0.64</td>
<td>3.3 ± 0.80</td>
<td>210</td>
</tr>
<tr>
<td>T. chebula</td>
<td>Fruit</td>
<td>1.5 ± 0.006</td>
<td>0.6 ± 0.002</td>
<td>40</td>
</tr>
</tbody>
</table>

(Values presented average of three determinations and expressed as Mean ± S. D)

(Enzymatic and non enzymatic levels of ethanolic plant extracts)

Catalase (CAT)

The catalase (CAT) activity was assayed by the titrimetric method. To 2.5 ml of phosphate buffer, pH 7.5, 2.5 ml of the 0.9% H₂O₂ (v/v) in the same buffer were taken and 0.5 ml of the enzyme extract was added and incubated at room temperature for 30 minutes. The reaction was carried out similarly with boiled enzyme extract. Unit of enzyme activity was expressed as ml of 0.1N KMn⁰₄ equivalents of H₂O₂ decomposed per mg protein.

Estimation of non-enzymatic antioxidants

Estimation of Vitamin – C

Ascorbic acid content was determined by the procedure described previously by. Pipette out 5.0 ml of the working standard solution in to a 100ml conical flask then 10ml of 4% oxalic acid was added and titrated against the dye. End point was the appearance of pink color, which persists for a few minutes. The amount of dye consumed is equivalent to the amount of ascorbic acid present in the plant extracts. Similar titration was carried out with 5.0 ml plant extracts. Amount expressed as (mg/100 g).

RESULTS

The results summarized in Table 1 among selected plants T. chebula shown significant antimicrobial activity. The plant showed highest (24 mm) activity against B. bicolor. C. asatica is significant against P. syringeae and E. carotovora (16 mm). S. xanthocarpum activity is excellent against E. carotovora (13 mm) B. bicolor, P. marginales and X. campesiris (12 mm). No activity was found with C. asatica, S. Xanthocarpum, T. chebula against C. herbarum.

The results obtained on enzymatic, non-enzymatic antioxidant levels are presented in Table 2: Among selected plants highest SOD activity (3.9 ± 0.64), significant CAT (3.7 ± 0.66), non-enzymatic antioxidant levels Vitamin – C (210 / 100 g) with S. xanthocarpum.

DISCUSSION

There is high demand for natural antioxidants in the food, cosmetic and therapeutic industry, due to their low cost, high stability, high compatibility. We found that medicinal plants are good sources of natural antimicrobial agents and antioxidants. The varia-
tion of susceptibility of the tested microorganisms could be attributed to their intrinsic properties that are related to the permeability of their cell surface to the extracts. Due to the emergence of antibiotic resistant pathogens in hospitals and homes, plants are being looked upon as an excellent alternate to combat the further spread of multidrug resistant microorganisms. The present study was conducted to develop newer lead for better and safer chemotherapeutic agents from medicinal plants. Further research is necessary for successful separation, purification and characterization of biologically active compounds using chromatographic methods and spectroscopic techniques. Further studies are needed to establish the exact mechanism of action for antimicrobial action of the plant extract. However, the strength of the existing data is not enough to suggest a reasonable mode of action for antimicrobial and antioxidant effects. The data of this study may just enrich the existing comprehensive data of biological activity.

REFERENCES


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