Twelve plant extracts extracted in Vidya herbs Pvt. Ltd., were studied for their antimicrobial potency against food borne pathogens viz. Emblica officinalis, Moringa oleifera, Terminalia chebula, Coffea arabica, Withania somnifera, Cinnamomum verum, Gymnema sylvestre, Morinda citrifolia, Curcuma longa, Theobroma cacao, Mucuna pruriens and Punica granatum against food borne pathogens. E. officinalis, Morinda citrifolia, W. somnifera, M. oleifera and P. granatum have proved the best plant extracts by inhibiting all the bacterial growth evidenced by wider and clear zones of inhibition. M. oleifera inhibited S. aureus, B. licheniformis, B. megaterium and L. acidophilus at 46.66, 33.33 and 19.99 mg/ml with zones of 17, 18, 17 and 17 mm respectively. B. licheniformis was sensitive when treated with W. somnifera at 40, 20, 10 and 5 mg/100μl concentrations and inhibition zone was 24.45, 23.5, 21.7 and 19.25mm, against S. aureus, B. licheniformis, B. megaterium and L. acidophilus, respectively. E. officinalis inhibited S. aureus even at less concentration i.e. 24mg/60μl with wider zone 33mm comparable to standard. M. citrifolia was found to inhibit all the organisms with wide and clear zones. A sensitive bacterium was S. aureus with wider zone of 30.5, 20.25, 16.25 and 12 mm at 40, 20, 10 and 5 mg/100μl respectively. SEM was performed in order to visualize the actual damage in treated fungi viz. Aspergillus niger and Aspergillus fumigatus. It is clear from the microscopic observation that the spores were totally emaciated with irregular size and shape when treated with extracts of the study. Thus, most of the plant extracts have shown the potential inhibitory activity against food pathogenic bacteria and could become promising natural antimicrobial agents with potential applications in neutraceutical/pharmaceutical industry for controlling the food borne pathogenic bacteria.

Keywords: Natural Cosmeceuticals, Neutraceuticals and medicinal plant, Food Borne Pathogens.
The plants used in the present exploration have a broad range of use to mankind. Table 1 reveals the same. Based on the literature the following plants were selected for the present investigation.

Table 1: Shows the uses of plants used in the present study

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant name / solvent extract</th>
<th>Family/ common name</th>
<th>Traditional uses and biological activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Emblica officinalis Lam. /water</td>
<td>Euphorbiaceae/ Amla</td>
<td>Valued in traditional Indian medicine, rich dietary source of vitamin C, minerals and amino acids and various phenolic compounds. Critical components in ‘triphala’ rasayana, anti-carcinogenic, hypoglycemic, hepatoprotective, anti-inflammatory, immunomodulatory, antidiabetes, antioxidant, anti-inflammatory and antipyretic. Neutriceutical applications like aphrodisiac, haemostatic, nutritive tonic, an excellent brain tonic, rejuvenative. In cosmetics it is used as hair tonic, anti-aging.</td>
</tr>
<tr>
<td>2</td>
<td>Moringa oleifera/100% Ethyl alcohol</td>
<td>Moringaceae/ horse radish</td>
<td>Good source of protein, amino acids vitamins, β – carotene and various phenolics, fatty acids, vitamins, and nutrients. Anti-inflammatory, hepatoprotective, antihypertensive and anti-cancerous. Antimicrobial properties antioxidant, antidiabetic, antiviral, anti-influenza. An outstanding detoxifier, outstanding immune builder and is used in many countries to treat malnutrition and malaria. hypo-tensive, antibacterial.</td>
</tr>
<tr>
<td>3</td>
<td>Punica granatum L./ water</td>
<td>Punicaceae/ pomegranate</td>
<td>Antimicrobial properties antiviral, antiparasitic, antitumor, AIDS.</td>
</tr>
<tr>
<td>4</td>
<td>Coffea Arabica /70% Methanol</td>
<td>Rubiaceae/ coffee</td>
<td>Influenza, anemia, edema, asthmasia and rage, hepatitis and liver troubles, externally for nervous shock, as a stimulant for sleepiness and drunkenness, as an antitussive in flu and lung ailments, as a carminative and a nervine tonic and for asthmatics. Coffee is a paliative in spasmodic asthma, in whooping cough, it is highly recommended in cholera infantum, chronic diarrhoea. Caffeine and coffee used in diuretic dropsy. Roasted coffee has disinfectant and deodorant effect.</td>
</tr>
<tr>
<td>5</td>
<td>Withania somnifera L. Dunal / water</td>
<td>Solanaceae/Ashwagandha</td>
<td>Mild laxative and astringent against wounds and abscesses. In the dental care dried powder is applied against stomatitis and against ulcers of the gum. It is excellent antidote against bites of snakes, antitussive, diuretic, homeostatic, laxative and cardiotonic treatments. Drug is a remedy against a sore throat and cough, against long during diarrhoea connected with a prolapsed rectum, ulcers and dysentery. Antibacterial, antiviral, antimutagenic, anti-cancer, antioxidant, cytoprotective, antispasmodic, gastrointestinal, immunosuppressive, antidiabetic, cardiotonic. Preventive against atherosclerosis.</td>
</tr>
<tr>
<td>6</td>
<td>Curcuma longa L./ Ethylene dichloride</td>
<td>Zingiberaceae/ turmeric</td>
<td>Reduces blood cholesterol prevents LDL oxidation inhibits platelet aggregation suppresses thrombosis and myocardial infarction (MI), suppresses symptoms associated with type II diabetes, rheumatoid arthritis, multiple sclerosis (MS), and Alzheimer’s disease, inhibits human immunodeficiency virus (HIV) replication, enhances Wound healing, protects from liver injury, protects from caftaract formation and an antihyperglycemic agent.</td>
</tr>
<tr>
<td>7</td>
<td>Gymnema sylvestre R.Br./ 90% methanol</td>
<td>Asclepiadaceae/</td>
<td>Antimicrobial, anti-hypercholesterolemic, antidiabetic activities anti-sweetener.</td>
</tr>
<tr>
<td>8</td>
<td>Cinnamomum verum/ Water</td>
<td>Lauraceae/ cinnamon</td>
<td>Antidiabetic, anti-nociceptive, astringent and diuretic activities; bark and leaves of C. verum contain antifungal substances, antioxidant allergic rhinitis, wound healing.</td>
</tr>
<tr>
<td>9</td>
<td>Mucuna pruriens (L) DC./ 1% Acetic acid in water</td>
<td>Fabaceae/ velvet bean</td>
<td>Carminative, hypertensive and hypoglycemic agent hyperglycaemic activity, antioxidant activity to increase testosterone levels; antibacterial activity; contains L-DOPA, a potent neurotransmitter precursor.</td>
</tr>
<tr>
<td>10</td>
<td>Terminalia chebula Retz. / 70% methanol</td>
<td>Combretaceae/ black myrobalan</td>
<td>Used in the treatment of asthma, sore throat, vomiting, hiccup, bleeding, piles, diarrhoea, gout, heart and bladder diseases. Black myrobalan has reported to have antidiabetic and free radical scavenging activities, antitumor, antihyperglycemic, and antibacterial. It used in dermal wound healing and improving gastrointestinal motility, antidiabetic activity.</td>
</tr>
<tr>
<td>11</td>
<td>Morinda citrifolia L./ Water</td>
<td>Morindaceae/noni</td>
<td>The leaves used to treat cough, nausea and colic, possibly due to its anti-inflammatory activity, gout, tuberculosis and ring.</td>
</tr>
</tbody>
</table>
MATERIALS AND METHODS
Preparation of extract dilution series
Dissolve the desired amount of plant extract in respective solvents, with glass beads, vortex to homogenize. As a precaution not to miss trace amounts of antibacterial for preliminary screening, a relatively high concentration of 50 to 400 mg/ml of each extract was prepared for bioassays.

Test organisms
The bacterial cultures used in this investigation were Staphylococcus aureus, Bacillus licheniformis, Bacillus megaterium and Lactobacillus acidophilus. To obtain cultures, the bacteria were inoculated into the nutrient broth and incubated overnight at 37°C. For bioassays, approximately 1.5x10^6 CFU/ml bacterial cell suspensions were prepared in sterile normal saline as described by Forbes et al. The fungal cultures Aspergillus niger and Aspergillus fumigatus were subcultured on PDA medium every 15 days to prevent pleomorphic transformations.

Agar well diffusion method
About 0.1 ml of each prepared bacterial suspension was uniformly seeded on sterilized nutrient agar petriplates. Left aside for 15 minutes and excess of suspension was then drained and discarded properly. Wells of 8 mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borer. As a precaution not to miss trace amounts of antimicrobials, a relatively punctured in the culture media using sterile cork borer. As a precaution not to miss trace amounts of antimicrobials, a relatively high concentration of 400, 200, 100 and 50 mg/ml of the extract was prepared in respective solvents and solution was administered in high concentration of 400, 200, 100 and 50 mg/ml of the extract was administered in each well. Culture plates were incubated 27°C for remaining all test bacteria. A positive control Amoxycillin (10 mg/ml) was used and negative control distilled water was used. After 24 h, bioactivity was determined by measuring diameter of inhibition zones (DIZ) in millimeter. All tests were performed in triplicate.

Minimum Inhibitory Concentration (MIC)
A primary factor in the decision to use an antibacterial drug in vivo is a minimum inhibitory concentration (MIC) of the drug in vitro, which suggests the target fungal species, is susceptible to that drug following the guidelines of The National Committee for Clinical Laboratory Standards (NCCLS) Subcommittee. The minimum inhibitory concentration was defined as the lowest concentration that substantially inhibited growth of the microorganism detected visually.

Agar Dilution Method (for fungi)
The in vitro antifungal activity of all the solvent extracts was done by poisoned food technique. In this method, the prepared test sample was dissolved in respective solvents, and the required amount was added to the cool molten potato dextrose agar which was then shaken vigorously, and poured into 9-cm diameter culture plates. The medium without extract served as control. Plugs of 1 mm 7 days old fungal culture cut from edge of active growing colony were stabbed at the centre of each petriplate and incubated at 37±2°C. A control set was also maintained as mentioned above for each experiment. The diameter of each fungus was measured after 3-7 days of incubation. Each experiment was done as triplicate. The percent mycelial growth inhibition of the test fungus was calculated as followed:

\[
I = \frac{C \times T}{C} \times 100
\]

Where,
\( I \) = percent mycelial growth inhibition
\( C \) = diameter of fungal colony in the control.
\( T \) = diameter of fungal colony in the treated.

Scanning Electron Microscopy
Fungal material obtained from cultures grown either in presence of plant extracts or in absence was processed for morphological studies at Indian Institute of Science, Bangalore, Karnataka, India. Conidial samples were recovered from 15 days old cultures. The morphological changes in the conidia of fungi grown in presence of various plant extracts were analyzed after comparing with control groups grown in control. All the photographs were captured at the same magnification i.e. X10,000.

RESULTS
The antimicrobial activity of twelve Plant extracts is depicted in the following figures (Fig.1, 2, 3, and 4). All the extracts have efficiently inhibited the organisms which are responsible for food spoilage. Of which, E. officinalis, Withania somnifera and Morinda citrifolia out performed a range of plants including G. sylvestre, P. granatum, C. arabica, C. longa, C. verum, T. cacao, M. oleifera, T. chebula and M. pruriens.

Antibacterial activity
E. officinalis inhibited S. aureus even at less concentration i.e. 5mg/100μl with wider zone 14mm. at 40 mg/100μl the results are comparable to standard Amoxycillin which inhibited S. aureus (33 mm). MIC of E. officinalis was 0.1875mg/100μl. M. oleifera inhibited S. aureus, B. licheniformis, B. megaterium and L. acidophilus at 46.66, 33.33 and 19.99mg/ml with zones of 17, 18, 17 and 17 mm, respectively. MIC of M. oleifera against S. aureus was 20.8mg/100μl (Table-2). C. arabica could not inhibited the growth of S. aureus, but B. licheniformis, B. megaterium and L. acidophilus were inhibited with zones of 12, 17 and 15mm, respectively. Similar results were obtained for the activity of P. granatum. T. chebula inhibited the growth of B. licheniformis and L. acidophilus with equal clear and wider zones i.e. 20, 19.5, 19 and 18mm at 40, 20, 10, and 5mg/100μl, respectively (Fig 1 and 4). MIC against S. aureus was 0.3125 mg/100μl. Curcuma longa had least activity, but MIC against B. megaterium was 0.3125 mg/100μl. B. licheniformis was sensitive when treated with W. somnifera at 40, 20, 10 and 5 mg/100μl concentrations and inhibition zone was 24.45, 23.5, 21.7 and 19.25mm. MIC against B. licheniformis was 1.25 mg/100μl (Table-2).

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Organism</th>
<th>MIC (mg/0.1ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. officinalis</td>
<td>S. aureus</td>
<td>0.1875</td>
</tr>
<tr>
<td>M. oleifera</td>
<td>B. licheniformis</td>
<td>2.08</td>
</tr>
</tbody>
</table>

Table 2: Showing MIC Values of Plant Extracts
G. sylvestre exhibited antimicrobial activity against only S. aureus (10.5mm), B. licheniformis (12mm) and L. acidophilus (14.75mm) at 40, 20, 10 and 5 mg/100μl concentration. The results are in agreement with the work of Deb Roy Saumendu et al., (2010). MIC was 0.625 mg/100μl (Table-2). Widest inhibition zone was found with S. aureus (17.25mm) treated with 20 mg/100μl of C. verum followed by L. acidophilus (12.25mm) and B. licheniformis (11.25mm) (Fig. 1&4). MIC was 0.625 100 mg/100μl. M. pruriens could not inhibit S. aureus, but inhibited B. megaterium, B. licheniformis and L. acidophilus at 40 mg/100μl with wider zones of 16, 13.5 and 12.25mm, respectively. MIC of M. pruriens was 10 mg/100μl. M. citrifolia was found to inhibit all the organisms with wide and clear zones. A sensitive bacterium was S. aureus with wider zone of 30.5, 20.25, 16.25 and 12 mm at 40, 20, 10 and 5 mg/100μl, respectively. Similar results are reported by Khuntia Tapas Kumar et al., (2010). No zones were found at 5 mg/100μl for B. licheniformis, B. megaterium and L. acidophilus. MIC was found to be 5 mg/100μl. T. cacao was found to have the least activity where only S. aureus could be found to be inhibited with zone of 11, 10, 9 mm at 40, 20, 10 mg/100μl. MIC was 4 mg/100μl (Table 2)
Scanning Electron Microscopy (SEM)

There were remarkable morphological changes in conidiophores of *A. niger* and *A. fumigatus*. There was a deformation of conidiophores treated with plant extracts in both *A. niger* and *A. fumigatus* causing dwindling of cell wall, where shrunken and collapsed conidiophores were observed, which might be due to cell fluid leakage. In control *A. niger*, spores have many conspicuous echinulates. Some spores of *A. niger* treated with Coffee extract are seen totally broken with severe changes and those treated with Amla extract were bulged of varying size within the cells. Lesions observed on the structure and rigidity on conidial wall. Similar alterations were observed with Amla, *T. chebula*, *M. oleifera* and Pomegranate extracts (Plate I).

The spores of *A. fumigatus* treated with Coffee extract were noticed with bulged abnormal spores after the 4th day of treatment. *T. chebula* has potently acted on *A. fumigatus* where ruptured, shrunken and dissimilar shaped spores were observed. Similar changes were seen in fungi treated with Pomegranate extract (Plate I).

PLATE I:

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**Fig. 3:** Antimicrobial activity of Vidya herbs plant extracts against *Staphylococcus aureus*

**Fig. 4:** Antimicrobial activity of Vidya herbs plant extracts against *Lactobacillus acidophilus*
Plate I: Scanning electron microscopy study of A. niger treated with various plant extracts. A= Control, spores with sharp points with a rough cell wall; B= spores completely broken thin, shrunken with severe changes treated with Coffee extract; C= broken spores treated with Amla extract; D & E= broken and emaciated spores treated with T. chebula and Pomegranate extract; F= slightly shrunken spores treated with M. oleifera extract.

DISCUSSION

Today, most pathogenic organisms are becoming resistant to antibiotics. Despite the huge information of uses of the plant extracts used in the present study, scanty literature is available on their use as preservatives in foods.

E. officinalis (Amla), Withania somnifera and Morinda citrifolia efficiently inhibited all the bacteria even at low concentrations. E. officinalis was found to be the potent inhibitor against all bacteria, but inhibited S. aureus to the largest extent with clear and wide zone when compared to other three bacteria. Similar studies were done by Sabahat and Perween54, where Amla infusion and decoction inhibited S. aureus with 18.32 and 22.45mm zone of inhibition at 20 mg/100μl. Similarly, work of Raghu and Ravindra55 depict that S. aureus was inhibited at 5, 10 and 20 mg/100μl concentration of Amla with zone of 8, 18 and 28mm. But, Vidya herbs amla extract of the present study has highest activity and more significant than the reports of the above authors. Even at low concentration i.e. 3 mg/100μl, it has inhibited S. aureus. This observation provides strong circumstantial evidence that E. officinalis has been used in the traditional method of treating a bacterial infection. These consequences suggest that E. officinalis Lam. used contain bio-components whose antibacterial potentials are highly comparable with that of the antibiotic Amoxicillin against all bacteria tested.

T. chebula is one of the major ingredient of ‘triphala churna’ and ayurvedic preparation used as health tonic, it plays a vital role in building immunity and also acts as a potent nutraceutical. In the present study, T. chebula (black myrobalan) has potentially inhibited all the organisms. Sumathi and Parvathi56 studied antimicrobial activity of T. chebula against S. aureus including other bacteria. They report that T. chebula did not inhibit any of the test organisms. But the report of the present study strongly oppose the results of the above authors, where in the present study T. chebula has substantially inhibited S. aureus even low concentration i.e. 5 mg/100μl with 15mm inhibitory zone.
PLATE II:

Plate II: Scanning electron microscopy study of *A. fumigatus* treated with various plant extracts.

A= Control, echinate spores with slight furrow; B= bulged spores of varying size treated with Coffee extract; C= shrunken and irregular shaped spores treated with Pomegranate extract; D= broken and more withered spores treated with *T. chebula* extract.

Pomegranate extract in the study of Gislene G.F Nascimento\textsuperscript{57} report that hydro alcoholic extract of pomegranate extract could not inhibit *S. aureus*. The present study is in accordance with the above authors. Pomegranate pericarp (Peel, rind) Phenolic punicalagins; gallic acid and other fatty acids; catechin, EGCG; quercetin, rutin and other flavonols; flavones, flavonones; anthocyanins\textsuperscript{58}. On the basis of the knowledge of these properties, will be possible to use of pomegranate peel extracts to formulate new products to be used in food industry as natural antioxidant, replacing synthetic antioxidants, and also as natural food reservatives and pharmacological studies.

Cinnamon extract showed inhibitory activity against all the test bacteria except *B. megaterium*. Highest activity was shown against *S. aureus*. *C. verum* extract did not inhibit *S. aureus* even at 500, 250 and 125 mg/ml. The extract in the present study exhibited strong inhibitory activity against *S. aureus*, one of the very common food pathogens. *M. citrifolia* (noni) extract has substantially inhibited all the bacteria in the present study. The results are comparable to the standard antibiotic Amoxycillin. It is noticed that the activity was even more than the standard against *S. aureus*.

Most of the Plants used in the present investigation contain phenolic acid, tannin, flavonoids etc. viz., Coffee, Amla, Pomegranate, Black myrobalan, Noni, Horse radish, Cocoa contain high amount of total polyphenols which are responsible for good inhibitory activity against food borne bacteria used in the present study, there by preventing various disease such as diabetes, skin related infections, wound healing, cancer etc. As a result, our report evidently proves that Vidya Herbs plant extracts can be exploited as potential preservatives in food. Thus they provide safe, easy, efficient and useful solutions for common diseases without any toxins leading to a pleasant, hygienic, ambiance.

SEM was performed in order to visualize the actual damage in treated fungi. It is clear from the microscopic observation that the spores were totally emaciated with irregular size and shape when treated with extracts of the study. SEM results supplement the *in vitro* antimicrobial activity studied.

The consistent inhibitory activity against food borne pathogens of the plant extracts used is concentration dependent. Few extracts have not shown inhibitory activity at even higher concentration and vice versa. They might have activity even at more high concentration.

Herbs and spices have been used for thousands of years to enhance the flavor, colour and aroma of food. In addition to boosting flavor, herbs and spices are also known for their preservative.

Thus, all the plant extracts of the present investigation have shown the potential inhibitory activity against food pathogenic bacteria and
could become promising natural antimicrobial agents with potential applications in nutraceutical/pharmaceutical industry for controlling the food borne pathogenic bacteria. Since most of the plants of the present study viz, Amla, Moringa, pomegranate, coffee, cocoa are well known food plants, nutraceuticals and cosmeceuticals as well, they can be explored as excellent food preservative which enhance the nutritive value of the food products. They also can be used as one of the ingredients in cosmetic cream preparation. It is suggested that further research should be conducted to test the preservative effect of the extracts on some food models and with other food borne pathogens. The interaction of plant extracts with different food models will be reported in our next communication. However, if plant extracts are to be used for medicinal purposes, issues of safety and toxicity are also to be considered.

REFERENCES


