ABSTRACT: The aqueous and methanolic extracts of spinach (Spinacea oleracia), pumpkin (cucurbita pepo), suran (Amorphophalus campanulatus) and ghuiya (Colocasia esculenta) were evaluated for antimicrobial activity against bacterial strains (Bacillus cereus, Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Enterobacter agglomerans, Salmonella enteritidis, Salmonella choleraeus, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Penicillium chrysogenum, Enterobacter faecalis, Klebsiella pneumonia, B.sphericus, B.thruengenesis and Cryptococcus meningitis). The in vitro antimicrobial activity was performed by Agar well diffusion method on Nutrient Agar medium and Muller Hinton Agar medium. The methanolic extracts of all the vegetables showed moderate to high activity against all the investigated microbial strains. Methanolic extract of spinach was most effective among all extracts (32mm inhibition zone against E. coli). Methanolic extracts were more effective than their aqueous extracts.

**Keywords:** Antimicrobial activity, aqueous extract, methanolic extract, Spinacea oleracea, Amorphophalus campanulatus, Colocasia esculenta, Cucurbita pepo.

INTRODUCTION:
The increasingly high numbers of bacteria that are developing resistance to classical antibiotics drive much of the current interest on natural antimicrobial molecules in hope that they may provide useful leads into anti-infective drug candidates. Several antimicrobial agents were isolated from plant including secondary metabolites as essential oil and terpenoids, amongst which can be cited xanthones, benzenophenones, coumarins and flavonoids (H.Belguith et al., 2010). These new chemical substances can also serve as templates for producing more effective drugs through semi-synthetic and total synthetic procedure. About 74% of 119 plant-derived pharmaceutical medicines or biotechnology medicines are used in modern medicine in ways that correlate directly with their traditional uses. On numerous occasions, the folkloric records of many different cultures have provided information of plants with useful medicinal properties (Miraj k. Chowdhury et al., 2009). Glycolipid fractions from spinach have been reported to suppress colon tumor growth in mice by inhibiting the activities of neovascularization and cancer cellular proliferation in tumor tissue (Noaki Maeda et al., 2008). Lars P. Christensen et al., 2008 had presented a review on the bioactivity and bioavailability of naturally occurring galactolipids as well as their distribution in vegetables and certain fruits. A renewed interest has occurred in the last decade to search for phytochemicals of native and naturalized plants for pharmaceutical and nutritional purposes with the recognition that plant-derived products have great potential as sources of pharmaceuticals. Although leaves, roots, flowers, whole plants, and stems were examined for useful phytochemicals in many research projects, few reports refer to seeds as, sources for pharmaceuticals.

In our current study we have studied the antimicrobial potential of spinach (Spinacea oleracia), pumpkin (cucurbita pepo), suran (Amorphophalus campanulatus) and ghuiya (Colocasia esculenta).
MATERIALS AND METHOD:

Samples:

Raw materials used in this work are freshly harvested vegetables such as Spinach (Spinacea olerasia), Ghuiya (Colocesia esculenta), Pumpkin (Cucurbita pepo), and Suran (Amorphophalus campanulatus) from a local farm in Lucknow.

Test Organisms Used:

The test micro-organisms used for the anti-microbial activity screening were B. cereus, B. subtilis, E. coli, E. aerogenes, E. agglomerans, S. enteritidis, S. cholerasuis, S. aureus, P. aeruginosa, C. albicans, P. chrysogenum, E. faecalis, K. pneumonia, B.sphericus, B.thruengenesis and C. meningitis species were obtained from the C.D.R.I. Lucknow. Each bacterial strain was suspended in Mueller-Hinton broth (Difco, France) and incubated at 37°C for 18 h. Mueller-Hinton Agar (MHA, Difco, France) was used for testing antibacterial activity.

Culture media, Antibiotics and Phytochemical Solution:

Muler Hinton Agar and Nutrient agar were used for agar well diffusion assay. Amoxicillien(10mcg, for gram +ve), Gentamycien(10mcg, for gram-ve),and Fluconazole (10mcg, for fungus) were used as positive control.

Preparation of Extract:

About 10gm of the selected vegetables were macerated or extracted with 70% methanol and distilled water in a conical flask at wrist action rotary shaker for 48 hours. The whole extract was then filtered with the help of whatman no. 4 filter paper. The collected solvent was then evaporated to dryness with rotary vaccum evaporator at 40 °C to afford a thick residue. The thick residue was then converted in dried powder in a dessicator. This dried mass was dissolved to get 20 mg/ml concentration of extract.

Antimicrobial Activity Test:

The Antimicrobial susceptibility testing was done by using the Agar well diffusion method to detect the presence of anti bacterial or anti fungal activities of the samples (R.C.Jagessar et al., 2008). The sterile Nutrient Agar media is poured into the Petri plates. As soon as the agar was solidified a well is prepared into the plates with the help of a borer that was sterilized with alcohol and flame. A sterile swab was used to evenly distribute bacterial or fungal culture over the surface of the sterile nutrient agar plates. The plates were allowed to dry for 15 minutes before use in the test. The test samples were introduced into the wells. The antibiotics were introduced into the centre of the well for control. The plates were labeled and incubated overnight at 37 °C. Microbial growth was determined by measuring the diameter of zone of inhibition. The diameter of inhibition zones was measured in mm and the results were recorded.
RESULTS AND DISCUSSION

In particular the antimicrobial action of each compound was different in intensity and specificity. The extract from Spinacea oleracea, Cucurbita pepo, Amorphophalus campanulatus and Colocesia esculenta shows the effective and determinative anti-microbial activity against the selected bacterial strains (Bacillus cereus, Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Enterobacter agglomerans, Salmonella enteritidis, Salmonella cholerasuis, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter faecalis, Klebsiella pneumonia, B. sphericus, B. thruengenesis, Cryptococcus meningitis and Penicillium chrysogenum). Amorphophallus campanulatus was extracted with aqueous and methanol.

Table 1 and Figures 1-4 present the results of antimicrobial activity against selected microbes.

Table 1: Zone of inhibition from vegetal extracts in mm

<table>
<thead>
<tr>
<th>Test organism used</th>
<th>Spinach</th>
<th>Pumpkin</th>
<th>Ghuiya</th>
<th>Suran</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqx</td>
<td>Mtx</td>
<td>Aqx</td>
<td>Mtx</td>
<td>Aqx</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>12</td>
<td>17</td>
<td>10</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>12</td>
<td>29</td>
<td>12</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>B. sphericus</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>Cryptococcus meningitis</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>10</td>
<td>32</td>
<td>15</td>
<td>23</td>
<td>10</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>22</td>
<td>18</td>
<td>16</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>Salmonella cholerasuis</td>
<td>15</td>
<td>16</td>
<td>14</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>A. niger</td>
<td>12</td>
<td>20</td>
<td>14</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>14</td>
<td>24</td>
<td>12</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Penicillium crysogenum</td>
<td>14</td>
<td>16</td>
<td>12</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

Figure-1: Antimicrobial activity of Spinach
Antimicrobial Activity of Suran

% yield of aqueous and methanol extracts for suran was found to be 0.54 (brown color mass) and 2.56 (reddish brown color mass) respectively. % purity of tannins in aqueous and methanol extracts was found to be 0.0052 ± 0.03 and 0.038 ± 0.35 respectively. Amount of tannins in methanolic extract was greater than aqueous extract. Therefore, crude tannins were isolated from methanolic extract and the yield was 0.002%. Crude extract of suran (20mg/ml) showed maximum activity against S. aureus (24mm) whereas the minimum activity is reported against Penicillium crysogenum (14mm).

Antimicrobial Activity of Spinach

Leaves of Spinach was extracted with aqueous and methanol. % yield of aqueous and methanol extracts were found to be 0.52 (blackish green color mass) and 2.51 (aqua green color mass) respectively. % purity of glycolipids in aqueous and methanol extracts was found to be 0.0050 ± 0.02 and 0.035 ± 0.34 respectively. Amount of glycolipid and glycoglycerolipid in methanolic extract was greater than aqueous extract. Therefore, crude tannins were isolated from methanolic extract and the yield was 0.002%. Crude extract of spinach (20mg/ml) showed maximum activity against the Escherichia coli (32mm), Staphylococcus aureus (29mm) zone of inhibition respectively whereas the minimum activity is reported against the Bacillus subtilis (12mm) and Penicillium crysogenum (15mm). Aqueous extract of spinach shows a minimum zone of inhibition against Escherichia coli (10mm), Staphylococcus aureus (12mm), Candida albicans (14mm), Bacillus subtilis (12mm), Klebsiella pneumonia (14mm) but we get maximum result of this extract against Penicillium chrysogenum (22mm).

Antimicrobial Activity of Pumpkin

The fruity body of Cucurbita pepo (pumpkin) was extracted with aqueous and methanol. % yield of aqueous and methanol extracts were found to be 0.51 (white yellow color mass) and 2.46 (yellow color mass) respectively. % purity of tannins in aqueous and methanol extracts was found to be 0.0053 ± 0.02.5 and 0.038 ± 0.33 respectively. Amount of linoleic acid in methanolic extract was greater than aqueous extract. Therefore, crude linoleic acid were isolated from methanolic extract and the yield was 0.001.5%. The crude methanolic extract of pumpkin shows the sufficient zone of inhibition against the microorganisms as 23 mm in Escherichia coli, 22 mm in Staphylococcus aureus and 20 mm in Klebsiella pneumonia respectively.

Antimicrobial Activity of Ghuiya

The corm of Colocasia esculenta (ghuiya) was extracted with aqueous and methanol. % yield of aqueous and methanol extracts were found to be 0.51 (off white color mass) and 2.46 (white color mass) respectively. % purity of tannins in aqueous and methanol extracts was found to be 0.0052 ± 0.02.8 and 0.037± 0.34 respectively. Amount of linoleic acid in methanolic extract was greater than aqueous extract. Therefore, crude linoleic acids were isolated from methanolic extract and the yield was 0.001.7%. The crude methanolic extract of ghuiya (20mg/ml) showed maximum zone of inhibition against the microorganism A.niger (22mm) wherwas the minimum activity is reported against candida albicans (16mm).
REFERENCES