PHOTOGRAPHICAL STUDY OF PHYTOCHEMICAL ANALYSIS AND IN VITRO ANTIMICROBIAL ACTIVITY OF THREE IMPORTANT VEGETABLES FROM BRASSICACEAE FAMILY

M. Satya Prasad, DSD Suman Joshi, K Narendra, SK Nadiya, SK Masthani, N Padmaja Phani, A Krishna Satya *
Department of Biotechnology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India

Received on: 27/05/15 Revised on: 11/07/15 Accepted on: 24/07/15

*Corresponding author
Dr. A Krishna Satya, Department of Biotechnology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India
E-mail: akrishnasatya78@gmail.com

DOI: 10.7897/2277-4343.066143

ABSTRACT
Phytochemical studies of various extracts from vegetables have recently been a great interest in the field of pharmacological research, because of their essentiality in the medicinal field to replace the synthetic antimicrobial drugs with natural products. The present study investigate the phytochemical screening, and in vitro antimicrobial study on ethanol, methanol and aqueous extracts of three vegetables leaves from Brassicaceae family. Leaves of three plants from the family Brassicaceae - were collected, dried and powdered. The powdered material was subjected to soxhlet extraction using various solvents and were allowed for evaporation. The crude extracts thus obtained were used for further investigation of phytochemical and antimicrobial activity. The results showed the presence of phytoconstituents and significant antimicrobial activity against the pathogens – Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhimurium, Salmonella paratyphi and Staphylococcus epidermis. Among these extracts ethanol extracts showed presence of high amount of secondary metabolites. The ethanol extract of cabbage showed the highest inhibitory activity against Bacillus subtilis. Antimicrobial activity of the above plants proved that ethanol is the most effective solvent for extracting broad spectrum antimicrobial compounds from selected plant. Antimicrobial nature of these plants could be useful to improve natural antimicrobial drug from vegetables.

Key words: Phytoconstituents screening, antimicrobial activity, pathogens, broad spectrum, plant origin.

INTRODUCTION
Phytochemicals are bioactive chemicals, synthesized naturally in all parts of the plant body 1. They are called as secondary metabolites because the plants that produce them may have little need for them. The quantity and quality of phytochemicals present in plant parts may differ from one part to another. Secondary metabolites are known to show curative activity against several elements in man, and other animals, therefore could explain the use of traditional medicinal plants for treatment of several diseases.

Brassicaceae vegetables are a good source of food around the world. Previously a diverse range of metabolites have been reported from this genus with regard to human nutrition. Extensive data is available on the biological activities of primary and secondary metabolites of Brassica plants, such as antioxidant, anticancer, and antimicrobial activity. Brassica oleracea var. capitata, Brassica oleracea var. botrytis, and R. sativus var. longipinnatus are the important members of Brassicaceae, well known as food and as a model systems for plant research.

MATERIALS AND METHODS

Plant materials
Brassicaceae family -Brassica oleracea Var. capitata (cabbage), Brassica oleracea var. botrytis (Cauliflower), and R. sativus var. longipinnatus (white radish) leaves were collected in month of August 2013 from out fields of Guntur district, Andhra Pradesh, India. The plants were identified and authenticated by a taxonomist.

These leaves were washed with distilled water, and shade dried till it is crisp (approximately 15 days). These dried samples were powdered and stored at 4°C until further use.

Extraction procedure
500 grams of plant powdered material was weighed and was subjected to Soxhlet extraction with three liters of the various solvents like Ethanol, Methanol and Water in successive mode respectively for 48 h (30 to 36 cycles). Various temperatures are maintained for each solvent extraction procedure – for ethanol extraction 40- 45°C, methanol extraction 45-50°C and water extraction 95-100°C. The solvent was recovered using Rotary Vacuum Evaporator and the concentrated extract was further evaporated to get dry powder. The dried powder was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation of Phytochemical screening, and Anti-microbial Evaluation.

Phytochemical analysis
1. Qualitative Analysis
Chemical tests were carried out on the Ethanol, Methanol, and aqueous, extracts using procedures to identify the phytochemicals as described by Sofowara2, Trease and Evans3 and Harborne4.

Test for Carbohydrates
To 2ml of extract, 1ml of Molisch’s reagent and few drops of concentrated sulphuric acid were added. Formation of Purple colour at the inter phase of the two layers indicated the presence of carbohydrates.
Test for Amino acids and Proteins
2ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple colour.

Test for Tannins
To 1ml of extract, 2ml of 5% ferric chloride was added. Formation of greenish black color indicated the presence of tannins.

Test for Saponins
To 2ml of extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer’s reagent were added. Presence of white precipitate indicated the presence of saponins.

Test for Flavonoids
5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of extract neutralized with addition of concentrated sulphuric acid. Appearance of yellow color indicated the presence of flavonoids.

Test for Alkaloids
To 2ml of extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Fehling’s solution A and B are added and heated for few minutes An orange red precipitate indicates the presence of alkaloids.

Test for Anthocyanin and Betacyanin
To 2ml of extract, 1ml of 2N sodium hydroxide was added and heated for 5minutes at 100°C. Formation of yellow color indicated the presence of betacyanin.

Test for Quinones
To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicated the presence of quinones.

Test for Glycosides
To 1 ml of the extract add few drops of HCl, allowed for 5 minutes for hydrolysis and neutralized with NaOH solution. A few drops of Fehling’s solution A and B are added and heated for few minutes An orange red precipitate indicates the presence of glycosides.

Test for Terpenoids
To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Red brown color formation at the interface indicated the presence of terpenoids.

Test for Phenols
To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of greenish black color indicated the presence of phenols.

Test for Coumarins
To 1 ml of extract, 1ml of 10% Sodium hydroxide was added. Formation of yellow color indicated the presence of coumarins.

II. Quantitative analysis
Estimation of Total Proteins
Total protein in the plant extracts was determined using colorimetric method described by O.H. Lowry, et.al, (1951) 7. Plant extract (0.4 ml) was mixed with 4 ml of copper sulphate solution and incubated at room temperature for 10 minutes. Then, 4 ml of phenol reagent was allowed to react for 30 minutes. The absorbance was measured at 600 nm against reagent blank. Bovine serum albumin (1mg/ ml) was used as standard and then 15, 30, 60, 90, 120 and 150 µg were taken from the standard solution and these readings were used to calculate the total amount of proteins.

Estimation of Total Sugars
Total sugar in the plant extract was determined by M. Dubois, et.al, (1956) 6. Plant extract (1 ml) was mixed with 1 ml of 2% phenol and 5 ml of concentrated sulphuric acid, allowed to react for 30 minutes and absorbance was measured at 430 nm against reagent blank. For total sugar estimation glucose (1mg/ml) was used as a standard and then 20, 40, 60, 80, 100 µg were taken from the standard solution and readings were used to know the total sugars present in extraction samples.

Antimicrobial activity by disc plate method
Authentic pure cultures of bacteria namely Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhimurium, Salmonella paratyphi and Staphylococcus epidermis were collected from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh.

Preparation of Discs
From the plant extracts, 100 mg of crude extracts were dissolved in 1 ml of 4 % dimethyl sulphoxide (DMSO) and 0.2 ml of the prepared extracts were loaded on to the filter paper discs (Sterilized Whatmann No. 1 filter paper discs of 6 mm diameter) to get 20 µg / disc concentration and allowed to dry at room temperature in laminar air flow chamber 7-10.

Preparation of media
Muller Hinton Agar (MH, Hi media) was used11. The component of MH agar (gm/litre) are Beef extract 2gm, casein acid hydrolysate 17.5g, starch 1.5 g and agar 17g; pH 7.4 ± 0.2. About 38g of MH agar was weighed and dissolved in 1000 ml of distilled water and adjusted to pH 7.4 ± 0.2, sterilized by autoclaving at 121 °C for 15 minutes at 15 psi pressure and was used for sensitivity tests 7-10.

Antimicrobial activity
The antimicrobial activity of the extracts was evaluated by disc diffusion method12. Previously prepared paper discs containing different extracts were placed individually on the surface of the petriplates, containing 20 ml of respective media seeded with 0.1 ml of previously prepared microbial suspensions individually (10 CFU/ml). Standard antibiotic Streptomycin (20 µg/disc) obtained from Hi-media, Mumbai, was used as positive controls. The discs containing aqueous, methanol and ethanol served as negative controls. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs. The plates were incubated for 24 h at 37°C and the diameter of the inhibition zones was recorded.
Table 1: Phytochemical screening

<table>
<thead>
<tr>
<th>Compound</th>
<th><strong>Brassica oleracea</strong> var. capitata</th>
<th><strong>Brassica oleracea</strong> var. botrytis</th>
<th><strong>R. sativus</strong> var. Longipinnatus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water extract</td>
<td>Methanol extract</td>
<td>Ethanol extract</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Anthocyanins &amp; β-Cyanins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Quinones</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2: Quantitative analysis of Proteins and Sugars

<table>
<thead>
<tr>
<th>Botanical name (Local name)</th>
<th>Extract</th>
<th>Total protein (in µg)/mg of extract</th>
<th>Total sugars (in µg)/mg of extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brassica oleracea</strong> var. capitata (Cabbage)</td>
<td>Aqueous extract</td>
<td>56.1 ± 96</td>
<td>89 ± 1.15</td>
</tr>
<tr>
<td></td>
<td>Methanol extract</td>
<td>162 ± 96</td>
<td>486.6 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>Ethanol extract</td>
<td>174 ± 1.92</td>
<td>514 ± 3.0</td>
</tr>
<tr>
<td><strong>Brassica oleracea</strong> var. botrytis (Cauliflower)</td>
<td>Aqueous extract</td>
<td>90.5 ± 2.54</td>
<td>107.3 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Methanol extract</td>
<td>156 ± 96</td>
<td>408.6 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Ethanol extract</td>
<td>165 ± 2.54</td>
<td>491.3 ± 3.0</td>
</tr>
<tr>
<td><strong>R. sativus</strong> var. Longipinnatus (White radish)</td>
<td>Aqueous extract</td>
<td>57.2 ± 96</td>
<td>45.3 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Methanol extract</td>
<td>159 ± 96</td>
<td>267.3 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>Ethanol extract</td>
<td>162 ± 96</td>
<td>480 ± 3.4</td>
</tr>
</tbody>
</table>

*Each value is presented as mean ± S.D. (n=3)

Figure 1: Antimicrobial activity of various extracts of *Brassica oleracea* var. capitata.
RESULTS

Phytochemical screening
Phytochemical screening on aqueous, methanol and ethanol extracts of *Brassica oleracea* var. *capitata*, *Brassica oleracea* var. *botrytis* and *R. sativus* var. *longipinnatus* showed the presence of carbohydrates, amino acids, tannins, flavanoids, phenols, proteins and coumarins. Flavanoids are present in all the above said extracts except in ethanol extract of white radish. Alkaloids are present in the ethanol extract. Aqueous and methanol extractions showed the presence of terpenoids. Quinones are found only in aqueous and ethanol extractions of the cabbage. Anthocyanins, β-Cyanins and Saponins are completely absent in above extract. (Table 1)

Quantitative analysis of Proteins and Sugars
The results of total protein content and sugars are given in table 2. The total proteins content was found to be more in ethanol extractions followed by methanol and aqueous extractions. The values are represented as the mean ± S.D. of three measurements.

Antimicrobial activity
Antimicrobial activity of various extracts of *Brassica oleracea* var. *capitata*, *Brassica oleracea* var. *botrytis* and
**R. sativus var. longipinnatus** graphical representation of results are given in figures 1, 2 and 3. These extractions exhibited distinct zones of inhibition towards bacterial strains like *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhimurium, Salmonella paratyphi and Staphylococcus epidermis*. Among the above extracts ethanol extracts showed a greater activity than the other extracts. Antimicrobial activity of these plants proved that ethanol is the most effective solvent for extracting broad spectrum of antimicrobial compounds from plant origin. The inhibitory zones of different extracts varied with the type of microorganism involved in the work. These inhibitory zones were compared with the standard antibiotic Streptomycin (20µg).

**DISCUSSION**

Phytochemical constituents such as tannins, flavonoids, alkaloids, phenols, terpenoids, coumarins and several other aromatic compounds are regarded as secondary metabolites of plants, which serve a defense mechanism against many microorganisms. The present study was carried out on the various plant extract to reveal the presence of medicinally active constituents such as carbohydrates, amino acids, tannins, flavonoids, alkaloids, phenols, terpenoids, quinines, proteins, and coumarins in most of the selected plants which could be responsible for the observed antimicrobial property (summarized in Table 1). Tannins bind to proline rich proteins and interfere with the protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms.

Many studies are available on antimicrobial, anti-inflammatory properties of plants which have suggested that these plants could be useful to improve natural antimicrobial agents by inhibiting the extra cellular enzymes, which are required for microbial growth, oxidative phosphorylation and other microbial metabolic activities. Phytochemicals of vegetables serves as supplement of food and provide a better protection against infectious agents. Phytochemical studies states that the complex mixture of phytochemicals in vegetables provides a better protective effect on health than single one.

In the present study the agar disc diffusion method was used to evaluate the antimicrobial activity by measuring the inhibition zone against the test microorganisms. The results obtained by various extracts of *Brassica oleracea* var. *capitata*, *Brassica oleracea* var. *botrytis* and *R. sativus* var. *longipinnatus* showed that ethanol extracts exhibited prominent antibacterial activity against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhimurium, Salmonella paratyphi and Staphylococcus epidermis*. Out of these extracts ethanol extracts showed that maximum inhibition zones against test organisms. These studies suggested that ethanol extracts of the plant leaves provide broad range antimicrobial activity against such organisms.

**CONCLUSION**

The present study reveals that the ethanol extracts of these vegetables possess number of secondary metabolites and also shows antimicrobial activity. Out of which ethanol extract of cabbage showed the highest inhibitory activity against *Bacillus subtilis*. Antimicrobial activity of the above plants proved that ethanol is the most effective solvent for extracting broad spectrum of antimicrobial compounds from plant origin. Antimicrobial nature of these plants could be useful to improve natural antimicrobial drug from vegetables.

**ACKNOWLEDGEMENT**

The authors acknowledge to NRK & KSR Gupta Degree College, Tenali, for providing platform for this work and thanks to the Department of Biotechnology, Acharya Nagarjuna University, Guntur for supporting to carry out this work.

**REFERENCES**

10. National Committee for clinical laboratory standards, performance standards for antimicrobial disc susceptibility testing, twelfth information supplement (M100-S2), Wayne, PA, NCCLS; 2002.

Cite this article as:

Source of support: Nil, Conflict of interest: None Declared

Disclaimer: IJRAP is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IJRAP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IJRAP editor or editorial board members.