Research Article

www.ijrap.net

A COMPARATIVE 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 phytometabolites 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: Phytometabolites screening, antimicrobial activity, pathogens, broad spectrum, plant origin.

INTRODUCTION

Phytochemicals are bioactive chemicals, synthesized naturally in all parts of the plant body¹. 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

I. 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 distilled water was added and shaken in a graduated cylinder for 15 minutes length wise. Formation of 1cm layer of foam indicated the presence of saponins.

Test for Flavonoids

5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of extract followed by 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 Mayer's reagent were added. Presence of white precipitate indicated the presence of alkaloids.

Test for Anthocyanin and Betacyanin

To 2ml of extract, 1ml of 2N sodium hydroxide was added and heated for 5 minutes 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)⁵. 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 chamber⁷⁻¹⁰.

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 tests⁷⁻¹⁰.

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 Himedia, 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

Compound	<i>Brassica oleracea</i> var. <i>capitata</i>			<i>Brassica oleracea</i> var. <i>botrytis</i>			<i>R. sativus</i> var. <i>Longipinnatus</i>		
	Water extract	Methanol extract	Ethanol extract	Water extract	Methanol extract	Ethanol extract	Water extract	Methanol extract	Ethanol extract
Carbohydrates	+	+	+	+	+	+	+	+	+
Amino acids	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	-
Alkaloids	-	-	+	-	-	+	-	-	+
Phenols	+	-	+	+	+	+	+	+	+
Terpenoids	+	+	-	+	+	-	+	+	-
Anthocyanins & β -Cyanins	-	-	-	-	-	-	-	-	-
Quinones	+	-	+	-	-	-	-	-	-
Proteins	+	+	+	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-	-

Table 2: Quantitative analysis of Proteins and Sugars

Botanical name (Local name)	Extract	Total protein (in μg)/mg of extract	Total sugars (in μg)/mg of extract
<i>Brassica oleracea</i> var. <i>capitata</i> (Cabbage)	Aqueous extract	$56.1 \pm .96$	89 ± 1.15
	Methanol extract	$162 \pm .96$	486.6 ± 2.3
	Ethanol extract	174 ± 1.92	514 ± 3.0
<i>Brassica oleracea</i> var. <i>botrytis</i> (Cauliflower)	Aqueous extract	90.5 ± 2.54	107.3 ± 1.1
	Methanol extract	$156 \pm .96$	368.6 ± 1.1
	Ethanol extract	165 ± 2.54	491.3 ± 3.0
<i>R. sativus</i> var. <i>Longipinnatus</i> (White radish)	Aqueous extract	$57.2 \pm .96$	45.3 ± 1.1
	Methanol extract	$159 \pm .96$	267.3 ± 2.3
	Ethanol extract	$162 \pm .96$	480 ± 3.4

*Each value is presented as mean $\pm$ S.D. (n=3) Figure 1: Antimicrobial activity of various extracts of *Brassica oleracea* var. *capitata*.

Figure 2: Antimicrobial activity of various extracts of *Brassica oleracea* var. *botrytis*.

Figure 3: Antimicrobial activity of various extracts of *R. sativus* var. *Longipinnatus*.

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 $\pm$ 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 microorganisms¹³. 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 synthesis¹⁴. 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-helminthes, and anti-inflammatory properties of plants¹⁵⁻¹⁹. Some of these observations have helped in identifying the possible principle of new drugs for the therapeutic use in human beings. A number of studies highlighted the potential importance of vegetables as source of medicine. Vegetable extracts are given as a solution for the treatment of microbial diseases²⁰. Phytochemical compounds obtained from the vegetables acts as antimicrobial agents by inhibiting the extra cellular enzymes, which are required for microbial growth, oxidative phosphorylation and other microbial metabolic activities²¹. The 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 one²².

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

1. P. Tiwari, B. Kumar, M. Kaur, G. Kaur, H. Kaur, Int. Pharm. Scientia, 2011; 1: 98-106.
2. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books, Ibadan; 1931.
3. Trease GE, Evans WC. Pharmacognosy 11th edition Brailiae Tiridal Can. Macmill and Publishers; 1989.
4. Harborne JB. Phytochemical methods. Chapman and Hall Ltd., London; 1973. p. 49-188.
5. O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall. "Protein measurement with the folin phenol reagent" J Bio Chem. 1951; 193: p. 265-75.
6. M. Dubois, K. A. Gills, J. K. Hamilton, P. A. Rebers, F. Smith. "Colorimetric method for determination of sugars and related substances" Anal. Chem. Washington, DC. 1956; 28: p. 350-356.
7. Agnese AM, Perez C, Cabrera JL. Adesmiaegiceras: antimicrobial activity and chemical study. Phytomedicine 2001; 8: 389-394. <http://dx.doi.org/10.1078/0944-7113-00059>
8. Acar JF and Goldstein FW, Disc susceptibility test: Antibiotics in laboratory medicine, 4th edition, edited by L. victor, Williams and Wilkins publishers; 1998.
9. Bauer AW, Kirby WMM, Sherris JC and Turck M, Antibiotic susceptibility testing, by a standardized single disc method, Am J Clinical pathology 1966; 45: 493-496.
10. National Committee for clinical laboratory standards, performance standards for antimicrobial disc susceptibility testing, twelfth information supplement (M100-S12), Wayne, PA, NCCLS; 2002.
11. C Baskaram, V Ratha bai, D Kanimozhi. Screening for Antimicrobial activity and phytochemical analysis of various leaf extract of *Murraya koenigii*. International Journal of Research in Ayurveda and Pharmacy 2011; 2(6): 1807-1810.
12. Bauer AW, Kirby WM, Sherris JC, Turch M. Antibiotic susceptibility testing by standardised single disc method. Amer. J. Clin. Pathology 1966; 45: 493-6.
13. Bonjar GHS, Nik AK, Aghighi S. Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions of Iran. J BiolSci 2004; 4: 405-412. <http://dx.doi.org/10.3923/jbs.2004.405.412>
14. Shimada T. Salivary proteins as a defense against dietary tannins. J. Chem. Ecol. 2006; 32 (6): 1149-1163. <http://dx.doi.org/10.1007/s10886-006-9077-0>
15. Samy, R.P. and S. Ignacimuthu, Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats in India. J. Ethnopharmacol. 2000; 69: 63-71. [http://dx.doi.org/10.1016/S0378-8741\(98\)00156-1](http://dx.doi.org/10.1016/S0378-8741(98)00156-1)

16. Palombo, E.A. and S.J. Semple. Antibacterial activity of traditional medicinal plants. J. Ethnopharmacol. 2001; 77: 151-157. [http://dx.doi.org/10.1016/S0378-8741\(01\)00290-2](http://dx.doi.org/10.1016/S0378-8741(01)00290-2)
17. Kumaraswamy, Y., P.J. Cox, M. Jaspars, L. Nahar and S.D. Sarker. Screening seeds of Scottish plants for antibacterial activity. J. Ethnopharmacol. 2002; 83:73-77. [http://dx.doi.org/10.1016/S0378-8741\(02\)00214-3](http://dx.doi.org/10.1016/S0378-8741(02)00214-3)
18. Stepanovic, S., N. Antic, I. Dakic and M. Svabicvlahovic. In vitro antimicrobial activity of propolis and antimicrobial drugs. Microbiol. Res. 2003; 158: 353-357. <http://dx.doi.org/10.1078/0944-5013-00215>
19. Bylka, W., M. Szafer-Hajdrych, I. Matalawskan and O. Goslinka. Antimicrobial activity of isocytoside and extracts of *Aquilegia vulgaris* L. Lett. Appl. Microbiol. 2004; 39: 93-97 <http://dx.doi.org/10.1111/j.1472-765X.2004.01553.x>
20. Sherman JM, Hodge HM. The bactericidal properties of certain plant juices. Journal of Bacteriology 1936; 31: 96 - 98.
21. Umamaheswari N, Rhama S, Kannahi M, Madhavan S. Antifungal activities of *Clitoria ternatea* leaf extract. Advances in Plant Sciences 2010; 23 (II): 377 –378.
22. Chu YF, Sun J, Wu X, Liu RH. Antioxidant and anti proliferative activities of common vegetables. Journal of Agricultural Food Chemistry 2002; 50: 6910 – 6914. <http://dx.doi.org/10.1021/jf020665f>

Cite this article as:

M. Satya Prasad, DSD Suman Joshi, K Narendra, SK Nadiya, SK Masthani,, N Padmaja Phani, A Krishna Satya. A comparative study of phytochemical analysis and *In vitro* antimicrobial activity of three important vegetables from Brassicaceae family. Int. J. Res. Ayurveda Pharm. 2015;6(6):767-772 <http://dx.doi.org/10.7897/2277-4343.066143>

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.