proceedings

MDPI

Proceedings

Comparative Antimicrobial Activity Study of *Brassica oleceracea* †

Sandeep Waghulde *, Nilofar Abid Khan *, Nilesh Gorde, Mohan Kale, Pravin Naik and Rupali Prashant Yewale

Konkan Gyanpeeth Rahul Dharkar College of Pharmacy and Research Institute, Karjat, Dist-Raigad, Pin code 410201, India; nileshgorde83@gmail.com (N.G.); kalemkpharm@gmail.com (M.K.); pravin.aazocom@gmail.com (P.N.); rupalikalp123@gmail.com (R.P.Y.)

* Correspondence: sandeepwaghulde@yahoo.com (S.W.); afnan.21@rediffmail.com (N.A.K.)

† Presented at the 22nd International Electronic Conference on Synthetic Organic Chemistry, 15 November–15 December 2018. Available Online: https://sciforum.net/conference/ecsoc-22.

Published: 14 November 2018

Abstract: Medicinal plants are in rich source of antimicrobial agents. The present study was carried out to evaluate the antimicrobial effect of plants from the same species as Brassica oleceracea namely, white cabbage and red cabbage. The preliminary phytochemical analysis was tested by using a different extract of these plants for the presence of various secondary metabolites like alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, steroids, carbohydrates, and amino acids. The in vitro antimicrobial activity was screened against clinical isolates viz gram positive bacteria Staphylococcus aureus, Streptococcus pyogenes, gram negative bacteria Escherichia coli, Pseudomonas aeruginosa. Extracts found significant inhibition against all the pathogens.

Keywords: plant extract; phytochemicals; antibacterial activity; antifungal activity

1. Introduction

Despite great progress in the development of medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. The impact is mainly observed in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance [1]. Plants are the richest source of natural antimicrobial agents. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics [2]. From ancient times, different parts of medicinal plants have been used to cure specific ailments [3]. Medicinal plants are widely used because of its easy availability and cost effectiveness. The active principles of many drugs found in plants are secondary metabolites. The antimicrobial activities of plant extracts may be found in a variety of chemical components, including aldehyde and phenolic compounds [4]. India is well known for Ayurveda, which is one of the important traditional medicines practiced. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented [5].

Brassicaceae vegetables are a good source of food around the world. In the past, a diverse range of metabolites has 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 species of Brassicaceae, these species are well known plant sources used as food as well as for plant research.

Brassicaceae or the Cruciferae family contains *Brassica oleracea*. This family is comprised of approximately more than 3400 described species distributed among 350 genera including

cauliflower, broccoli, kohlrabi, kale, cabbage and Brussels sprouts. Many researchers have highlighted the potentials of Brassica species as a source of antibacterial [6–8] substances.

Red cabbage is the member of the Brassicaceae family. It is a cool season cruciferous vegetable. Red cabbage (*Brassica oleracea var. capitataf. rubra*) is type of cabbage, also well known as purple cabbage, blue kraut, or red kraut and is widespread in the Mediterranean region. Red Cabbage is a herbaceous, biennial, dicotyledonous flowering plant. Its leaves are red or purple in colour and are normally consumed as coleslaw, salad and beverage [9–11].

2. Materials and Methods

2.1. Plant Materials

Brassica oleracea var. capitataf. rubra leaves were collected in month of December 2016 from local market of karjat, District - Raigad, Maharashtra, India. The plants were identified and authenticated by a taxonomist.

Red cabbage leaves were shade dried followed by hot air oven drying at 50 °C and then ground to a fine powder and stored in airtight container for the analysis. Fresh red cabbage leaves were grinded in the mixer for the collection of juice.

2.2. Preparation of Extracts

A 5 g of crushed vegetable samples was added to three different flasks and extracted using 60% methanol, 60% ethanol or 60% acetone with 1-min nitrogen flushing at 20 psi. The concentration of solvent was decided on the basis of available literature (Zhao & Hall, 2008; Cox et al., 2010). Flasks were kept in a shaking incubator (Innova 42, Mason Technology, Dublin, Ireland) at 100 rpm and 40 °C for 2 h. The infusions were filtered with Whatman #1 until a clear extract was obtained.

2.3. Phytochemical Analysis

2.3.1. Qualitative Analysis

Chemical tests were carried out on the Ethanol, Methanol, and aqueous, extracts using procedures to identify the phytochemicals as described by Sofowara [2], Trease and Evans3 and Harborne4[12–16].

Test	Procedure	Observation
Test for Carbohydrates	To 2 mL of extract, 1 mL 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	2 mL of filtrate was treated with 2–5 drops of ninhydrin solution placed in a boiling water bath for 1–2 min.	It was observed for the formation of purple colour.
Test for Tannins	To 1 mL of extract, 2 mL of 5% ferric chloride was added.	Formation of greenish black color indicated the presence of tannins.
Test for Saponins	To 2 mL of extract, 2 mL of distilled water was added and shaken in a graduated cylinder for 15 min length wise.	Formation of 1 cm layer of foam indicated the presence of saponins.
Test for Flavonoids	5 mL 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 Mayer's Test	To 2 mL of extract, 2 mL of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added.	Presence of white precipitate indicated the presence of alkaloids

Wagner's test	To a few mL of filtrate, few drops of Wagner's reagent were added by the side of the test tube.	A reddish –brown precipitate confirmed the test as positive.
Test for Anthocyanin and Betacyanin	To 2 mL of extract, 1 mL of 2 N sodium hydroxide was added and heated for 5 min at 100 °C.	Formation of yellow color indicated the presence of betacyanin.
Test for Quinones	To 1 mL of extract, 1 mL 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 min 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 0.5 mL of extract, 2 mL 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 1 mL of the extract, 2 mL 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, 1 mL of 10% Sodium hydroxide was added.	Formation of yellow color indicated the presence of coumarins.
PhytosterolLibermann–buchard's test:	The extract (50 mg) was dissolved in 2 mL acetic acid anhydride. To this, one or two drops of concentrated sulphuric acid were added slowly along the side of the test tube.	An array of color changes showed the presence of phytosterols.
Salkowski's test	The extract was treated with Salkowski's reagent	The yellowish colour with green fluorescence appearance indicated the presence of phytosterol in it.

2.3.2. Quantitative Analysis

Estimation of Total Proteins

Total protein in the plant extracts was determined using the colorimetric method described by O.H. Lowry, *et. al*., (1951) [5]. Plant extract (0.4 mL) was then mixed with 4 mL of copper sulphate solution and incubated at room temperature for 10 min. Then, 4 mL of phenol reagent was allowed to react for 30 min. The absorbance was measured at 600 nm against reagent blank. Bovine serum albumin (1 mg/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

The 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 min and absorbance was measured at 430 nm against reagent blank. For total sugar estimation glucose (1 mg/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 is done by disc plate method. Authenticed pure cultures of bacterias namely; *Escherichia coli*, *Bacillus subtilis*, *Candida Albicans*, *Psudomonas. Vulgeris* and *Aspergilus Niger* were collected.

Nutrient Agar preparation:

Ingredients:

1. 0.5% Peptone—this provides organic nitrogen
2. 0.3% beef extract/yeast extract—the water-soluble content of these contribute vitamins, carbohydrates, nitrogen, and salts
3. 1.5% agar—this gives the mixture solidity
4. 0.5% Sodium Chloride—this gives the mixture proportions similar to those found in the cytoplasm of most organisms
5. Distilled water—water serves as a transport medium for the agar's various substances
6. pH adjusted to neutral (6.8) at 25 °C.

Preparation:

These ingredients are combined and boiled for approximately one minute to ensure they are mixed and to sterilize them. Then they are cooled to around 50 °C (122 °F) and poured into Petri dishes thatare covered immediately. Once the dishes hold solidified agar, they are stored in refrigerated until used.

Procedure (Cup and Plate method)

1. Nutrient agar is poured into Petri dishes and allowed to solidify.
2. Then bacterial culture is spread on the plate.
3. Allow it settle for 10 min.
4. With the help of borer (6 mm diameter) two wells are prepared; marked it A and B
5. Sample solution A and B are prepared, about 1 mL is poured in respectively.
6. Allow it to stand for 15 min.
7. Then incubate it in incubator for 24 h.
8. Measure the zone by using antibiotic zone reader.

Minimum inhibitory concentration (MIC):

Purple cabbage extract solution was diluted with ethanol to 4, 2, 1, 0.5, 0.25 mg/mL, in sterile Petri dishes were added to 1 mL of different concentrations of diluents then poured into 20 mL culture medium, mixed thoroughly, after cooling and solidification take 1.1 mL (0.5 the McIntosh turbidity) of the test bacterial suspension, observed in an incubator for 24 h, each repeated three times, take average [10].

Result and Discussion

In the present study the comparison between the phytochemical property of red cabbage powder and juice were estimated. The preliminary phytochemical investigation on red cabbage powder and juice extracts revealed the presence of various secondary metabolites such as alkaloids, glycosides, steroids, flavonoids, saponnin, tannin, terpenoids and phytosterols in the different extracts (Tables 1 and 2).

There was a significant difference in the activity of the Methanolic extracts on the individual standard strains studied (Table 3). A mild inhibition with mean zones of 13.1 mm, 11.7 mm, 13.9 mm 12.9 mm and 11.4 mm for, respectively were recorded. Inhibitory activity was the same for S. Enteritidis 13,076 and *S. aureus* 29,213 on separation of treatment means. The highest growth inhibition was observed on *S. aureus* and lowest on E. coli strains.

Table 1. Preliminary Phytochemical Screening of Red Cabbage Powder Extract.

Name of the Chemical test	Methanol Extract	Distill Water Extract
Alkaloids	Present	Present
Gylcosides	Absent	Present
Steroids	Present	Present
Flavonoids	Present	Present
Saponin	Absent	Present
Tannin	Present	Present
Terpenoids	Absent	Present
Phytosterols	Present	Present

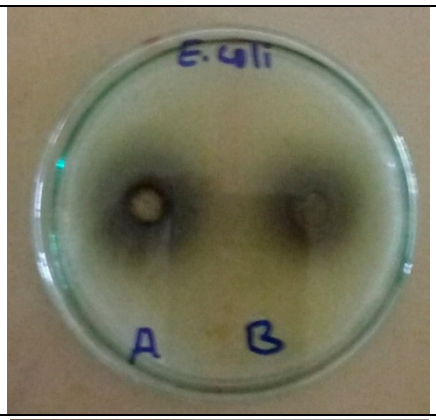
Table 2. Quantitative analysis of Proteins and Sugars.

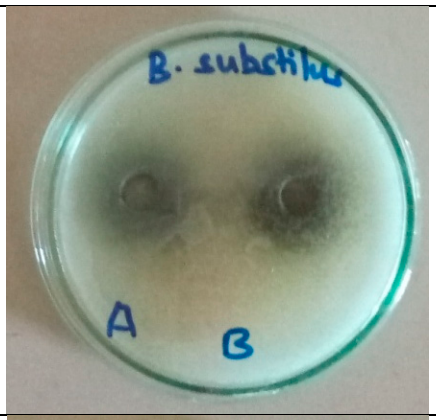
Botanical Name	Extract	Total Protein (µg)/mg of Extract	Total Sugar (µg)/mg of Extract
Brassica oleracea Brassica oleracea var. capitata*f. rubra*	Methanolic extract	165 ± 1.46	472 ± 1.24
	Distill water extract	52.3 ± 1.21	87 ± 2.21

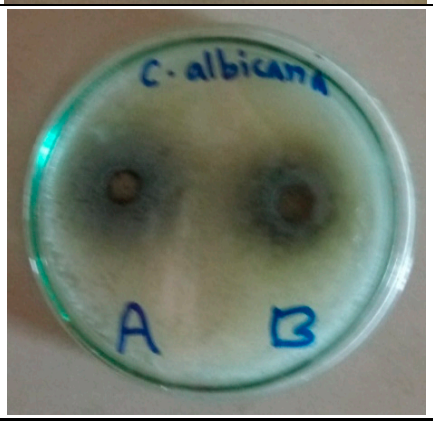
*Each value is presented as mean ± S.D. (n=3).

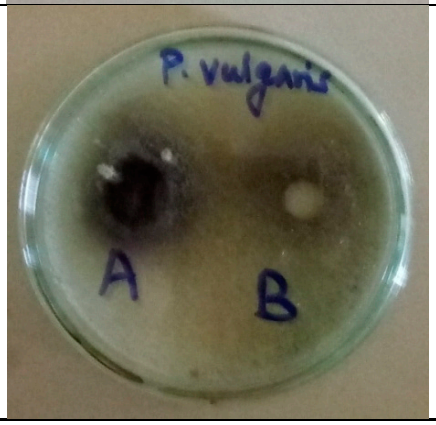
Table 3. Comparative study of antibacterial activities of Red Cabbage.

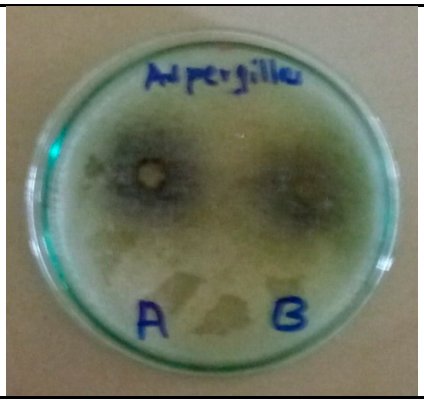
Extractive Solvent	Test Bacteria				
	Escherichia Coli	Candida Albicans	Bacillus Subtilis	P. Vulgeris	A. Niger
(A) Methanol extract	12.5 mm	11.7 mm	13.9 mm	6.0 mm	6.7 mm
(B) Distill water extract	8.5 mm	7.7 mm	7.9 mm	6.2 mm	6.2 mm











3. Discussion

The present study was carried out on the 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 plants, which could be responsible for the observed antimicrobial property (summarized in Table 1). 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. 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 showed that ethanol extracts exhibited prominent antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Candida Albicans*, *Psudomonas Vulgeris* and *Aspergilus Niger*. 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.

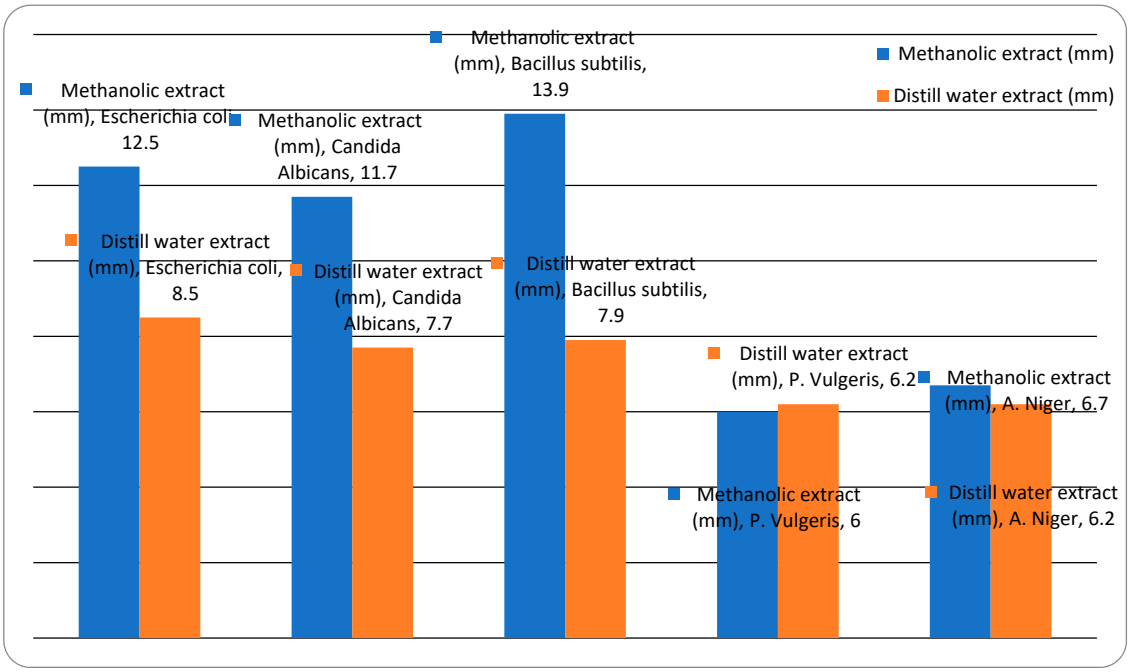


Figure 1. Antimicrobial activity.

4. Conclusions

The present study reveals that the ethanol extracts of these vegetables possess a number of secondary metabolites and also shows antimicrobial activity. Out of which the methanol 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 drugs from vegetables.

Acknowledgments: The authors acknowledge to Mohan Kale, Principal KGRDCP & RI Karjat College for providing platform for this work and thanks to the Department of Biotechnology for supporting to carry out this work.

References

1. Zampini, I.C.; Cuello, S.; Alberto, M.R.; Ordóñez, R.M.; D'almeida, R.; Solorzano, E.; Isla, M.I. Antimicrobial activity of selected plant species from "the Argentine Puna" against sensitive and multi-resistant bacteria. *J. Ethnopharmacol.* **2009**, *124*, 499–505.
2. Mathur, A.; Singh, R.; Yousuf, S.; Bhardwaj, A.; Verma, S.K.; Babu, P.; Gupta, V.; Prasad, G.B.K.S.; Dua, V.K.Antifungal activity of some plant extracts against clinical pathogens. *Adv. Appl. Sci. Res.* **2011**, *2*, 260–264.
3. Dubey, R.; Dubey, K.; Sridhar, C.; Jayaveera, K.N. Human vaginal pathogen inhibition studies on aqueous, methanolic and saponins extracts of stem barks of Ziziphusmauritiana. *IJPSR* **2011**, *2*, 659.
4. Lai, P.K.; Roy, J. Antimicrobial and chemopreventive properties of herbs and spices. *Curr. Med. Chem.* **2004**, *11*, 1451–1460.
5. Dubey, N.K.; Kumar, R.; Tripathi, P. Global promotion of herbal medicine: India's opportunity. *Curr. Sci.* **2004**, *86*, 37–41.
6. Kyung, K.H.; Fleming, H.P. S-Methyl-L-Cysteine sulfoxide as the precursor of methyl methanethiolsulfinate, the principal antibacterial compound in cabbage. *J. Food Sci.* **1994**, *59*, 350–355.
7. Hu, S.H.; Wang, J.C.; Kung, H.F.; Wang, J.T.; Lee, W.L.; Yang, Y.H. Antimicrobial effect of extracts of Cruciferous vegetables. *Kaohsiung J. Med. Sci.* **2004**, *20*, 591–599.
8. Ayaz, F.A.; Hayırlıoglu-Ayaz, S.; Alpay-Karaoglu, S.; Grúz, J.; Valentová, K.; Ulrichová, J.; Strnad, M. Phenolic acid contents of kale (Brassica oleraceae L. var. acephala DC.) extracts and their antioxidant and antibacterial activities. *Food Chem.* **2008**, *107*, 19–25
9. Lynn, A.; Collins, A.; Fuller, Z.; Hillman, K.; Rateliffe, B. Cruciferous vegetables and colorectal cancer.*Proc.Nutr. Soc.* **2006**, *65*, 135–144.
10. El-Mowafy, E.M.; Maha, A. Treatment Effect of Red Cabbage and Cysteine against Paracetamol Induced Hepatotoxicity in Experimental Rats. *J. Appl. Sci. Res.* **2012**, *8*, 5852–5859.
11. Chauhan, E.S.; Tiwari, A.; Singh, A. Phytochemical screening of red cabbage (Brassica oleracea) powder and juice—A comparative study. *J. Med. Plants Stud.* **2016**, *4*, 196–199, ISSN 2320-3862.
12. Handa, S.S. Quality control and standardization of herbal material and traditional remedies. *East Pharma* **1995**, *38*, 23–25.
13. Sofowora, A. *Medicinal Plants and Traditional Medicine in Africa*; Spectrum Books: Ibadan, Nigeria, 1931.
14. Trease, G.E.; Evans, W.C. *Pharmacognsy*, 11th ed.; Brailliae Tiridal Can; Macmill and Publishers: London, UK, 1989.
15. Harborne, J.B. *Phytochemical Methods*; Chapman and Hall Ltd.: London, UK, 1973; pp. 49–188.
16. Gogo, L.A.; Shitandi, A.A.; Lokuruka, M.N.I.; Sang, W. Antimicrobial Effect of Juice Extract from Fermented Cabbage Against Select Food-Borne Bacterial Pathogens. *J. Appl. Sci. Res.* **2010**, *6*, 1807–1813.