

Comparative Antimicrobial Activity study of *Brassica oleracea*

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Abstract:

Medicinal plants are in rich source of antimicrobial agents. The present study was carried out to evaluate the antimicrobial effect plants from same species *Brassica oleracea* namely, white cabbage and red cabbage. The preliminary phytochemical analysis was tested by using 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 were found significant inhibition against all the pathogens.

Keywords: Plant extract, Phytochemicals, Antibacterial activity, Antifungal activity.

INTRODUCTION

Despite great progress in 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]. The 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 important traditional medicine 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. 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 species of

Brassicaceae, these species are well known plant sources used as food as well as for plant research.

Brassicaceae or Cruciferae family contains Brassica oleracea. This family 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 Brassicaceae family. It is a cool season cruciferous vegetable. Red cabbage (*Brassica oleracea* var. *capitata* f. *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]

MATERIALS AND METHODS

Plant materials

Brassica oleracea var. *capitata* f. *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° centigrade and then ground to a fine powder and stored in air tight container for the analysis. Fresh red cabbage leaves were grinded in the mixer for the collection of juice.

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.

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 Sofowara[2], Trease and Evans³ and Harborne⁴. [12-15]

Name of the Test	Procedure	Observation
Test for Carbohydrates	To 2ml of extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were	Formation of Purple colour at the inter phase of the two layers indicated the presence of

	added.	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.	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 15minutes 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 Mayer's Test	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
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 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.
Phytosterol Liebermann– buchard’s test:	The extract (50 mg) was dissolved in 2ml 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.

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 is done by disc plate method. Authenticated pure cultures of bacterias namely; *Escherichia coli*, *Bacillus subtilis*, *Candida Albicans*, *Psudomonas. Vulgeris* and *Aspergillus 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 which are 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; allowed it to solidify.
2. Then bacterial culture is spread on the plate.
3. Allow it settle for 10 minutes
4. With the help of borer (6 mm diameter) two wells are prepared; marked it A and B
5. Sample solution A and B about 1ml is pour in the respectively.
6. Allow it to stand for 15 minutes.
7. Then incubate it in incubator for 24 hours.
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 20ml culture medium, mixed thoroughly, after cooling and solidification take 1.1mL (0.5 the McIntosh turbidity) of the test bacterial suspension, observed in an incubator for 24h, 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 (Table 1 &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* 13076 and *S. aureus* 29213 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

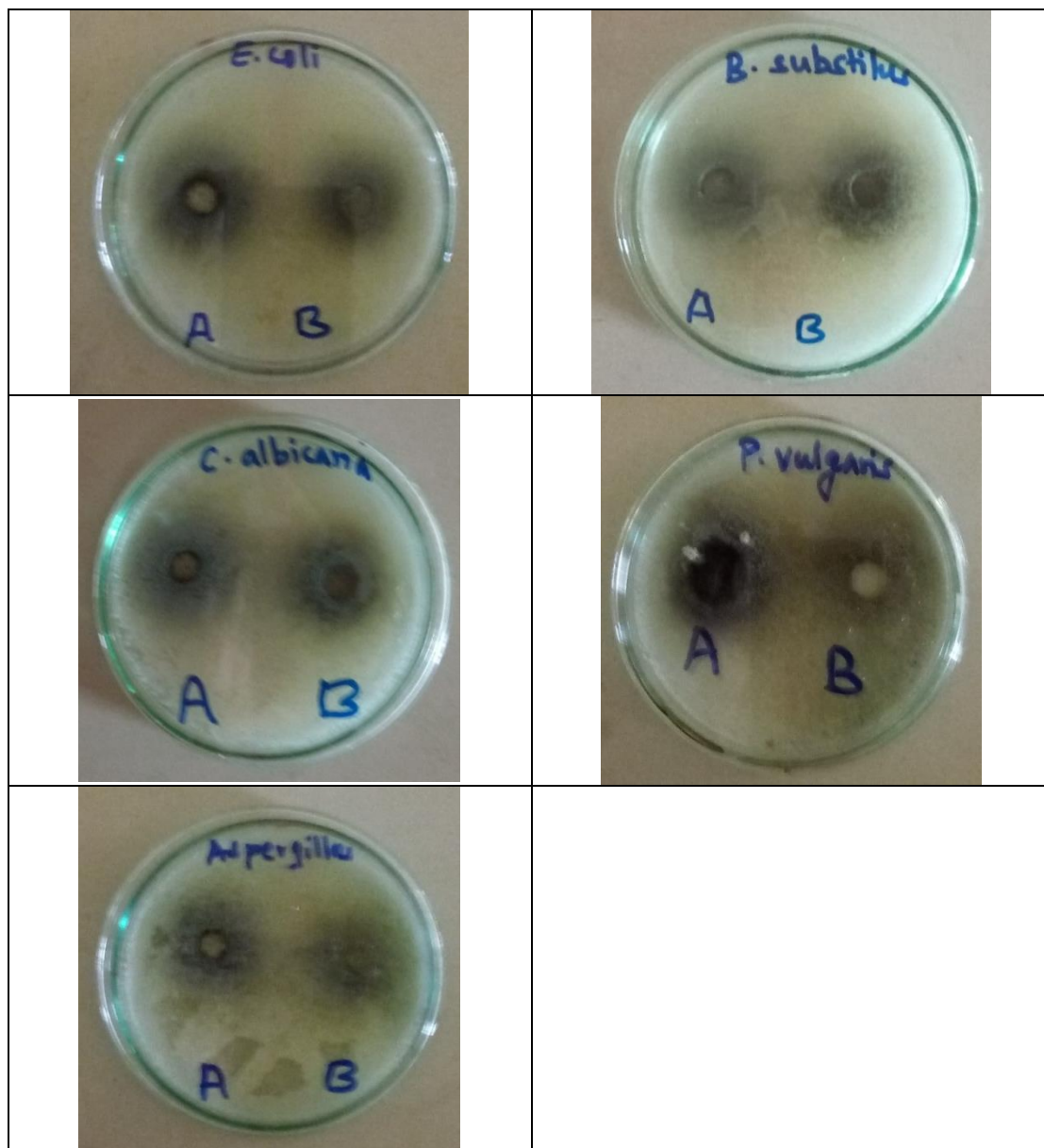
Table 2: Quantitative analysis of Proteins and Sugars

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

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

Table 3: Comparative study of antibacterial activities of Red Cabbage

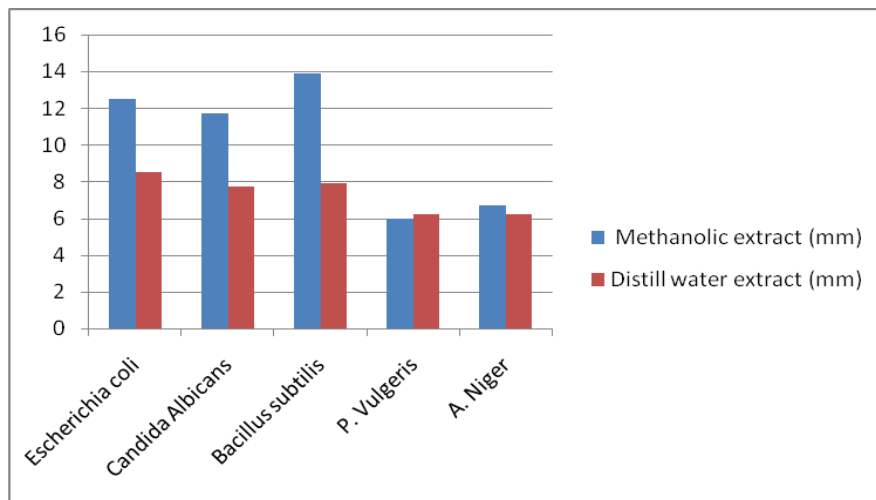
Extractive Solvent	Test Bacteria				
	Escherichia coli	Candida Albicans	Bacillus subtilis	P. Vulgeris	A. Niger
(A) Methanol extract	12.5 mm	11.7 mm	13.9 mm	6.0 mm	6.7 mm
(B) Distill water extract	8.5 mm	7.7 mm	7.9 mm	6.2mm	6.2 mm



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 were 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 *Aspergillus 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.



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 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 drug from vegetables.

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