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Evaluation of Antimicrobial Activity of *Brassica juncea* Leaves against Different Strains of Bacteria

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ABSTRACT
A large number of plants possess medicinal properties due to which they are used as therapeutic agents or an active ingredient of a medicinal preparation. A sizeable fraction of the population throughout the world depends on traditional practices to fight against various infections and diseases and nearly all of it involves the use of plant extracts gathered from a vast diversity of plants which may include Herbs, Shrubs, Trees, Climbers or Creepers. In the present work, we have attempted to study different characteristics of Brassica juncea leaves in different solvents including ethanol, methanol and distilled water, to understand its antimicrobial and antioxidant activity. Brassica juncea comes under the family Cruciferae and genus Brassica, is an important Rabi season oilseed crop. Various tests were carried out to estimate phytochemicals like tannins, flavonoids, phenols, alkaloids, glycosides, terpenoids, amino acids, steroids, anthroquinones, and saponins. Methanol extract of plant leaves were came out to be abundant in phenols and flavonoids. The antioxidant activity of B. juncea was determined by DPPH scavenging activity and it is revealed that methanol extract of this plant leaves possess maximum antioxidant activity. Antibacterial potential of plant leaves extracts were determined in vitro by the help of agar well diffusion method against Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia and Escherichia coli. Results were also compared with control (Piperacillin/Tazobactam). All the extracts of B. juncea leaves showed appreciable antibacterial activity.

KEYWORDS: *Antimicrobial activity, Antioxidant activity, Brassica juncea, Extracts, Phytochemicals.*

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1. INTRODUCTION

Plant and plant parts are well known for their medicinal effects since ancient times (Tiwari, *et al*, 2016). Antimicrobial and antioxidative properties of various plants parts have received appreciable attention in recent years (Sivasankaridevi, *et al*, 2013). In India and many other countries, the conventional methods of treatment by the use of natural products plays a very noteworthy task in medicinal management of human beings from countryside areas for various types of ailments (Taid, *et al*, 2014). About 400 different tribes and other ethnic groups resides in countryside areas of India and to a great magnitude they depend on native system of medicines produced from forests (Dutta, *et al*, 2005).

Antioxidants rich plants and their products play an indispensable role in the prevention of cancers, cardiovascular

diseases, neurodegenerative diseases and various inflammatory problems (Sukanpak, *et al*, 2012). Pharmacological activity of various plants or their products is a characteristic of various biologically active molecules such as sterols, aromatic acids, polyphenols, monoterpenoids, flavonoids, iridoids etc. existing in them (Nguelefack, *et al*, 2008).

All over the world, the considerable reason behind various treatment failure is the appearance of resistant bacteria, the outcome of which is the unsuccessfulness of present drugs and antibiotics (Djeussi, *et al*, 2013). This is the rationale behind the increasing attentiveness in using natural medicine and utilization of natural compounds obtained from plants as curatives (Abew & Sahile, 2014). Microbial cells are influenced by the plant secondary metabolites in a number of ways including interruption of nucleic acid synthesis and function, disruption of membrane function and structure, interruption of normal cell communication, induction of coagulation of cytoplasmic components and interference with intermediary metabolism (Radulonic, *et al*, 2013). Phenolic compounds showed powerful antioxidative effects, consequently their supplementation may aid to prevent oxidative damage, like that which happens in oxidation of lipid in association with premature ageing and cancer (Dimo, *et al*, 1999).

Brassica juncea is also called by other names such as Chinese mustard, leaf mustard, Indian mustard or green mustard and it comes under Brassicaceae (Cruciferae) family (Kumar, *et al*, 2011). It is grown throughout in India, mainly in Bengal, Bihar and Uttar Pradesh. It is an annual, branched, large crop growing up to 1 meter. The flowers of *Brassica juncea* are bright yellow in colour, stalked and in corymbose racemes. The fruits, Siliqua are 2.5-5cm in length, with linear oblong shape. Seeds of *Brassica juncea* are brownish-red in colour and spherical in shape (Alqasoumi, 2010).

The *Brassicaceae* family turn out to be a focus of rising interest because of its potential to lower the chances of various types of cancers especially lung cancer, gastrointestinal and bladder cancer (Abbaoui, *et al*, 2018; Navarro, *et al*, 2011). The potential of cruciferous plants to lower the risk of such deadly diseases is attributed to the carotenoids, fibers, vitamin C, and folic acid present in them along with Sulphur-containing secondary metabolites such as glucosinolates, which are also the reason behind their spicy or bitter taste (Ishida, *et al*, 2014). Keeping in view the edible and medicinal importance of *Brassica juncea,* the present study is aimed on the identification of various active phytochemicals present in *Brassica juncea* leaves with the aid of various chemical tests, detection of antioxidant activity existing in *Brassica juncea* leaves using DPPH method, and finally determination of antibacterial activity present in *Brassica juncea* leaves against various strains of bacteria using agar well diffusion method.

2. MATERIALS AND METHODS

Plant Material and Preparation of Extract

Brassica juncea leaves were collected in the month of February, 2019 from a local market in Ghaziabad, India. The collected fresh leaves were thoroughly cleaned under running water and kept in shade to dry them. The leaves were transformed into a powdered form using mixer grinder after they were completely dried. Ten gram of the plant leaves powder was wrapped into a blotting paper and put in a soxhlet apparatus which was then heated on a heating mantle with 80% of ethanol and methanol separately as well as with 200 ml of distilled water at temperature corresponding to the used solvents boiling points for 7-8 hours. As soon as the extraction process was completed, the liquid extracts were evaporated on a water bath at 100°C until the semi-solid extracts were produced. 25, 50, 75 and 100mg from each extract were weighed and each was resuspended in 1ml of respective solvent and dissolved with the help of Dimethyl sulfoxide (DMSO). The extracts were kept and stored at 4°C for further studies (Tiwari, *et al*, 2018).

Phytochemical Screening

Phytochemical screenings were done for the identification of present secondary metabolites of plants which are

responsible for the antimicrobial activity (Tiwari, *et al*, 2016; Harborne, 1998 and Kokate, 2001).

Test for Phenols and Tannins
Crude leaves extract was added to 2 ml of 2% $FeCl_3$ solution. Appearance of bluish green colour is the indicator for the presence of phenols and tannins.

Test for Flavonoids
Crude leaves extract was added to 2 ml of 2% NaOH solution. Appeared intense yellow colour became colourless after the addition of one to two drops of diluted acid which specifies the existence of flavonoids.

Test for Saponins
Crude leaves extract was added to 5 ml of distilled water and shaken vigorously. The appearance of stable foam indicated the presence of saponins.

Test for Glycosides
Liebermann's test: Crude leaves extract was added to 2 ml of chloroform and 2 ml of acetic acid, the mixture was kept on ice for cooling. Carefully H_2SO_4 (concentrated) was put into the tube. Change in colour from violet to green is the indicator for the existence of glycosides.

Test for Steroids
Crude leaves extract was added to 2 ml of chloroform into which carefully added the concentrated H_2SO_4. The presence of steroids is indicated by the red coloured layer formed at lower part of test tube. Alternative test was done by the addition of crude extract to 2-3 ml of Chloroform followed by the addition of 2 ml each of acetic acid and concentrated H_2SO_4. The presence of steroid confirmed with the appearance green colour.

Test for Terpenoids
Crude leaves extract was added to 2 ml of chloroform and then evaporated. To this mixture, 2 ml of concentrated H_2SO_4 was mixed and then warmed up for about 2-3 minutes. Terpenoids presence was confirmed by the appearance of grayish colour.

Test for Alkaloids
Crude leaves extract was added to 1-2 ml of 1% solution of HCl and then gently heated. To the mixture, Mayer's reagent was then dissolved. The presence of alkaloids was confirmed by the formation of turbidity in the resulting precipitate.

Test for Amino Acids
Few drops of the Ninhydrin reagent was added to the 1 ml of crude leaves extract. The presence of amino acids is confirmed by purple colour appeared in test tube.

Test for Anthraquiones
Diluted Sulphuric acid (H_2SO_4) was mixed with 5 ml of the crude leaves extract followed by the addition of dilute ammonia and benzene solution to it. Presence of anthraquinones is confirmed by the pink colour appeared in the test tube.

Determination of Total Phenolics
Folin-Ciocalteu colorimetric method was used for the determination of Total Phenolic Content (TPC) present in sample (Singleton, 1999), including some variations (Dewanto, 2002). Readings were obtained with diluted extract within the standard curve ranges of 0-600 µg of gallic acid/ml. 0.5-1 ml of distilled water was mixed with 120-125 µl of the standard gallic acid solution in a test tube then 120 µl of Folin-ciocalteu reagent was dissolved into it. The test tube was shaken well and the mixture was allowed to stand for 5-7 min followed by the addition of 1.25 ml of 7% aqueous sodium carbonate solution. Final volume was adjusted to 3 ml by the addition of water. Samples were kept at room temperature for 90 minutes followed by the measurement of absorbance at 760 nm versus the blank using a spectrophotometer in comparison with the standard solution prepared in the same way using known concentrations of gallic acid. All values were expressed in units of mg gallic acid equivalents (GAE) /100g extract).

Determination of Total Flavonoids
Colorimetric method was used to determine the Total flavonoid content (Dewanto, *et al.*, 2002). 1.25 ml of distilled water and 0.25 ml of diluted extracts were mixed together followed by the addition of 0.075 ml of 5% sodium nitrite solution

and allowed to stand for 7 min. After that 0.1 ml of 10% aluminium chloride solution was dissolved into it and allowed to stand for 8 min followed by the addition of 0.5 ml of 1 M sodium hydroxide solution to it. Final volume of the mixture was adjusted to 3 ml by the addition of distilled water. At 510 nm wavelength, absorbance of the mixture was measured against a prepared blank. Quercetin standard curve was used to determine the flavonoid content and expressed in terms of milligrams of Quercetin equivalents per g of extract.

Evaluation of free radical scavenging activity by DPPH method

Free radical scavenging capacity of the inspected plant extracts is measured by this assay (Molyneux, 2004). DPPH is a chemical compound that contains a stable free radical. DPPH can accept electrons donated by an antioxidant which results in the disappearance of purple colour typical of free DPPH radical followed by the measurement of absorbance change at λ = 517 nm. Based on the scavenging effect on the DPPH free radical activity, antioxidant potential of the leaf extracts were examined. 0.1 mM of DPPH methanol solution was prepared, from which 1ml is mixed with 3ml of each diluted extract and vitamin C taken as standard. For 30 minutes, the mixtures are kept at room temperature in dark followed by the measurement of at the wavelength 517 nm wavelength against a blank. The percentage radical scavenging effect of the extract is measured by given equation.

$$\text{Scavenging activity (\%)} = \frac{100 \times (Ao - As)}{Ao}$$

Where
Ao = blank absorbance;
As = sample absorbance.

Antimicrobial Activity

The micro-organisms *Escherichia coli* (MTCC1234), *Staphylococcus aureus* (MTCC1144), *Klebsiella pneumoniae* (MTCC4030) and *Bacillus subtilis* (MTCC1133) were collected from the Codon Biotech Laboratory Noida, U.P. The medium used for the estimation of antibacterial activity was Nutrient Agar Medium (NAM). NAM was prepared and sterilized in autoclave at 15psi pressure and 121°C for 15-30 minutes. Under laminar air chamber, petri plates of 90mm diameter are filled with 25ml of pre autoclaved NAM which were then allowed to solidify for 15-20 minutes. After that, 0.1ml of each microbial culture were spread over NAM containing plates with help of sterilized L-shaped spreader. Using a borer of 6mm diameter, wells were then made in each petri plate. 0.1 ml of each leaves extracts was then loaded into these wells. Well diffusion method is based on the diffusion of concerned extracts from the well within agar gel in the petri plate such that the growth of bacteria is completely prevented in this circular zone surrounding the well that contain extract. Petri dishes containing bacteria and leaves extract were incubated in an incubator at 37 °C for 24hrs. After that measurements of the clear zone diameter was done in mm and then compared with the standard drug used.

Statistical Analysis

The data are expressed as the mean ± standard deviation (SD). The statistical difference and significance among various groups was calculated through t-test by using GraphPad PRISM version 5.01 software. The values $p < 0.05$ considered as statistically significant.

3. RESULTS AND DISCUSSION

In the current study, *Brassica juncea* plant leaves are screened for their phytochemical constituents as well as antimicrobial and antioxidant potential. Quantitative analysis of Total Flavonoid Content (TFC) and Total Phenolic Content (TPC) in the plant leaves is also done.

Phytochemicals

After the completion of various tests carried out for the qualitative estimation of phytochemicals present in *B.juncea* leaves, it was found that all the extracts are rich in Phenols, Tannins, Flavonoids, Alkaloids, Amino acids and Saponins while Glycosides and Steroids were detected only in methanol and ethanol extract. Terpenoids were detected only in ethanol extract and Anthroquinones were not detected in any of the extract (Table 1).

Phytochemical testing of different extracts revealed that *B. juncea* leaves have considerable proportion of important phytochemicals that were easily detected by qualitative tests. In our analysis, it was established that ethanol extract of *B.juncea* leaves are abundant in phenols, steroids, terpenoids, alkaloids, and amino acids. The important thing is that all extracts contain two common and abundant secondary metabolites, phenols and flavonoids. Flavonoids have broad spectrum therapeutic effects such as antiviral, antibacterial, antiallergic, antitumor. Alkaloids and phenolic compounds along with antidiabetic properties also exhibit antimicrobial, antioxidant and anti-inflammatory effects. Moreover, saponins exhibit various biological activities like, it gives permeability to the cell membrane, helpful in lowering the serum cholesterol levels. Saponins also show antidiabetic characteristics (Zheng *et al.*, 2012; Oraon *et al.*, 2020). Tannins are reported to have anti-inflammatory, cardio-protective, anticarcinogenic and antimutagenic properties (Tungmunnithum *et al.,* 2018). Quinones inhibit HIV1 reverse transcriptase and shows antitumor and immunomodulatory activities (Valderrama *et al.,* 2003).

Table 1: Phytochemicals present in different extracts of *B. juncea* leaves.

Brassica juncea			
Test name	**Methanol extract**	**Ethanol extract**	**Aqueous extract**
Phenols	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Glycosides	+	+	-
Steroids	+	+	-
Terpenoids	-	+	-
Alkaloids	+	+	+
Amino acids	+	+	+
Anthroquinones	-	-	-

(+) Present (-) Absent

Total Phenol Content in *B. juncea* Extracts

Total phenol content was detected by FCR. As standard compound, Gallic acid was used and total phenols were measured in terms of mg GAE/100g extract. The chemical test carried out for the quantitative determination of phenolic content in *B. juncea* revealed that methanol extract has maximum phenol content with a concentration equivalent to about 37mg Gallic acid/100g of extract while ethanol extract and aqueous extract have somewhat equal phenol content with a concentration equivalent to about 32 mg Gallic acid/100g of extract (Figure 1).

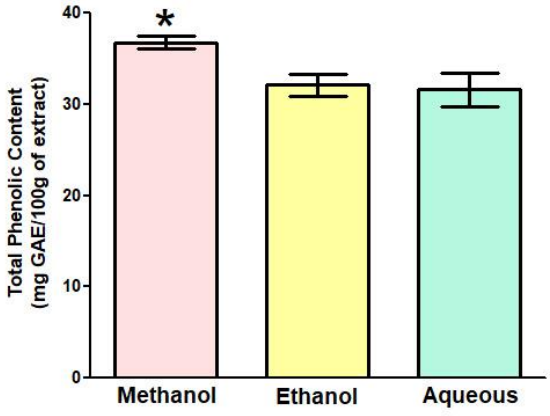

Figure 1: Total phenol content in *B. juncea* leaf extracts. Phenolic content is reported in terms of Gallic Acid Equivalents (GAE) mg/100g of the extract. Values are expressed as Mean ± SD of of three independent experiments. *p< 0.05 when compared with aqueous.

Total Flavonoid Content in *B. juncea* Extracts

For the determination of total flavonoid content, standard compound used was Quercetin and total flavonoids were measured in terms of mg QE/g extract. The phytochemical Flavonoid was found in maximum amounts in the Methanol extract of *Brassica juncea* leaves with a concentration equivalent to about 34mg of Quercetin/100g of extract. Ethanol extract and aqueous extract have similar levels of Flavonoids with a concentration equivalent to about 26-27 mg of Quercetin/ 100 g of extract (figure 2).

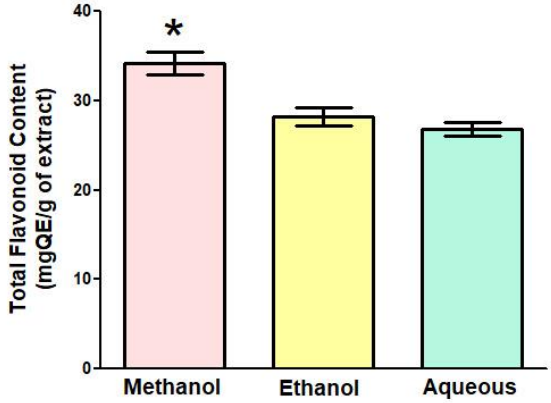

Figure 2: Total flavonoid content in *B. juncea* leaf extracts. Flavonoid content is reported in terms of Quercetin Equivalents (mg) /gram of the extract. Values are expressed as Mean ± SD of the three independent experiments. *p< 0.05 when compared with aqueous.

Antioxidant Activity

Antioxidant potential of the plant species is expressed as percentage DPPH scavenging activity. Methanol extract has high DPPH scavenging ability which means that methanol extract of *B.juncea* leaves has strong antioxidant in nature, which is attributed to the presence of phenols and flavonoids.

The DPPH assay for the determination of Antioxidant activity present in different extracts of *B. juncea* revealed that methanol extract has maximum antioxidant activity with 89% DPPH scavenging activity. Aqueous extract has minimum antioxidant potential with 73% DPPH scavenging activity. Ethanol extract has 81% DPPH scavenging activity (figure 3).

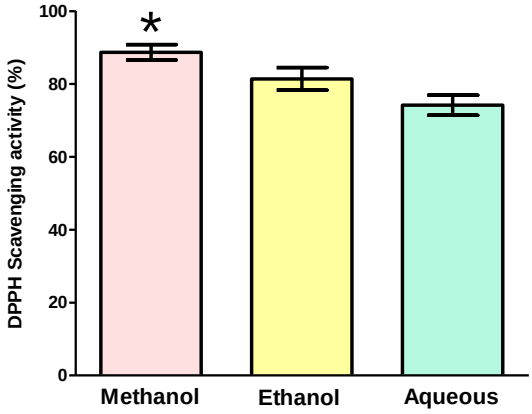

Figure 3: Percentage DPPH Scavenging activity of *B. juncea* leaf extracts. Values are expressed as Mean ± SD of the three independent experiments. *p< 0.05 when compared with aqueous.

Antibacterial activity

The antimicrobial activity of methanol, ethanol and aqueous extracts of *B.juncea* were analyzed in-vitro by the method called agar well-diffusion against two gram positive bacterial strains and two gram negative bacterial strains. The range of concentration used was 25 to 100mg/ml. The Antibacterial potential of leaves extract against each bacterial strain was found to be diverse. The inhibition zones of the bacterial strains were measured in the diameter range from 4mm to 22mm. Different species of microorganisms were differently influenced by the leaves extract depending on the concentration and type of extracts used. Results revealed that on elevating the concentration of extracts, zones of inhibition also enlarges. Researchers revealed that antibiotic activity of the natural products are due the presence of phytochemicals which has an ability to inhibit the growth of microorganism (Nascimento *et al.,* 2000; Tan *et al.,* 2015).

Antibacterial activity was observed in all the extracts of *B. juncea* leaves. Ethanol and methanol extracts of *Brassica juncea* gave comparatively much appreciable results as compared to the aqueous extract of *Brassica juncea* (Table 2).

Table 2: Antibacterial assay of *B.juncea* leaf extracts by agar well-diffusion method and comparison with control.

Diameter of zone of inhibition (mm)													
	Ethanol extract (mg/ml) *Brassica juncea*				**Methanol extract (mg/ml) *Brassica juncea***				**Aqueous extract (mg/ml) *Brassica juncea***				**Piperacillin/ Tazobactam (Control)**
	25	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**100/10 mcg/disc**
Escherichia coli	8	13	16	20	12	15	16	19	4	6	7	8	22
Klebsiella pneumonia	10	10	11	12	11	12	12	13	7	9	9	12	18
Staphylococcus aureus	12	16	18	20	10	16	16	18	11	13	13	15	20
Bacillus subtilis	11	12	13	16	10	11	12	15	6	7	7	8	16

4. CONCLUSION

The outcomes of the current study reveals the medicinal importance of the edible plant in consideration and indicates that a large number of plant species exists that contains compounds with antibacterial properties that can be utilized in the preparation of drugs to fight against various disease causing pathogens. Disease causing pathogens develop resistance to the chemical antibiotics after a certain period of time, this is the reason behind the use of plant products as better substitute as curatives. In the current investigation, it is disclosed that *Brassica juncea* leaves possess considerable antimicrobial and antioxidant properties. *B.juncea* has capacity to be used as a cost-effective antioxidant and antimicrobial agent against microbes which have evolved multi drug resistance. However, further investigations regarding the analysis of biologically important compounds present in different plant species can provide a natural alternative for the treatment of numerous diseases and infections caused by inspected microbes.

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