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PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY STUDIES OF LEAF OF *BRASSICA JUNCEA* VAR. RUGOSA THROUGH SEQUENTIAL SOLVENT EXTRACTION

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Keywords:

Brassica juncea var. *rugosa*, Phytochemical, Polyphenol compounds, Phenols, Flavonoids, Tannins, Antioxidant, Peroxidase

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ABSTRACT: Objectives: To find out different qualitative and quantitative phytochemical properties, it's *in-vitro* antioxidant properties and peroxidase activity of *Brassica juncea* var. *Rugosa* (record tro- 50197928) with fresh and sequential solvent extracts. **Methods:** The leaf sample of *Brassica juncea* var. *rugosa* was collected from Manipur and the sample was dried. The dried powder was extracted using Soxhlet with the sequential solvent of non-polar to polar. Preliminary phytochemical analysis for proteins, carbohydrates, phenols, alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, and quantitative estimation for total proteins, carbohydrates, alkaloids, phenols, flavonoids, and tannins. *In-vitro*, antioxidant activity was analyzed by using DPPH assay. Peroxidase activity was also checked. **Results:** The plant extracts showed the presence of a considerable amount of total alkaloids, total phenols, total flavonoids, total tannins, total proteins, total carbohydrates. The plant extracts also showed high antioxidant and peroxidase activity. **Conclusions:** From the study, we can conclude that the plant *Brassica juncea* var. *rugosa* has many important secondary metabolites and it has a potential for strong antioxidants.

INTRODUCTION: The giant-leafed mustard, also known as "Japanese mustard" or 'Head mustard'', *(B. juncea* var. rugosa) belonging to the family Cruciferae has broad reddish-purple variegated leaves. It is an essential vegetable crop in Manipur. It is commonly known as 'Hangam' in Manipur. It is mainly grown in winter season as kitchen garden crops in most of the houses of Manipur, India. Usually the plant grows up to 60 cm in height. The plant requires cold climatic conditions and well-drained moist soil. It can grow in semi-shade or no shade [1].

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In Manipur, hangam have been consumed by the people from time to time for its special flavor, odor, and nutritive value. It is mainly cultivated for the purpose of local consumption and selling in local markets [2].

Phytochemicals are biologically active compounds which are produced by plants through primary or secondary metabolism which generally help the plants in defense mechanisms against diseases or pathogen, and they are also beneficial to human health in various ways [3]. They are mostly found in plant parts like fruits, leaves, vegetables, and seeds. Recent studies suggested that the consumption of plants which are rich in polyphenols will help in the prevention against the development of various cardiovascular diseases, neurodegenerative diseases, cancer, and diabetes [4]. The order of polarity is very important in selecting the solvents for sequential solvent extraction.

It should have proceeded from non–polar solvents like hexane or petroleum ether to polar solvent, and water must be the last solvent [5]. It also relies on compound nature, if the compound which we wish to extract is polar in nature, it is liked to pre-treat with no polar solvents to remove non-polar compounds so as to refine our compound or products [6].

An antioxidant is a substance which delays or inhibits oxidative damage to a target cell, tissues or molecules [7]. An antioxidant has ability to scavenge the free radicals. The free radicals can also be scavenged by synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT) but they are restricted by legislative rules because they are believed to have some toxic side effects and as possible carcinogens. Good source of natural antioxidants are fruits and vegetables [8]. Brassica vegetables are believed to have more antioxidant properties as compared to other vegetable crops. Peroxidase in plants helps in catalyzing the reduction of hydrogen peroxide (H_2O_2) to water, making it non-toxic. H_2O_2 is a strong oxidizing agent; they are commonly the end products of metabolic reactions and are toxic when present in excess.

The main activity of peroxidases is removing this excess H_2O_2 from plants. They are also involved in the scavenging of Reactive Oxygen Species (ROS) [9]. The present study was aimed to evaluate the phytochemicals present in the plant extracts and also to know the potential of antioxidant activity.

MATERIALS AND METHODS:

Collection of Plant Samples: The plant samples were collected from Singjamei market in Imphal East, a local market in Imphal, Manipur. The fresh leaves were washed under running tap water, shade dried at room temperature, and powdered for further qualitative and quantitative analysis. Some of the fresh samples were stored in 4 ºC.

Extract Preparation: The powdered plant sample were extracted successively with petroleum ether, chloroform, ethyl acetate, methanol and water using Soxhlet apparatus at 50-80 ºC for 6-8 h (each solvent) in order to obtain polar and non-polar extracts. For each solvent extraction, the powdered pack was air-dried and used. The solvents of the respective extraction were stored at 4 ºC for further use.

Preliminary Qualitative Phytochemical Analysis: Different preliminary qualitative phytochemical analysis (Alkaloids, flavonoids, phenols, saponins, steroids, tannins, terpenoids, carbohydrates, proteins) was carried out to identify the secondary metabolites present in the various solvent extracts of the plant leaf sample [10, 11].

Quantitative Estimations:

Estimation of Total Proteins: Total protein was estimated using Bradford methods with slight modifications [12]. 0.5 g of leaf sample was extracted with 10 ml of phosphate buffer (ice cold) and centrifuged at 10,000 rpm for 10 min and the supernatant was taken. Take 0.2 ml of the supernatant and add 0.8 ml of distilled water and 5ml of diluted Bradford reagent and read the absorbance at 595 nm. A standard calibration curve was prepared with known concentrations of BSA (µg/ml).

Estimation of Total Carbohydrate Content: Phenol-sulphuric acid method for total carbohydrate [12]. Weigh 0.1 g of the leaf sample and make an aqueous solution of it into a boiling tube. Hydrolyze by keeping it in boiling water bath for carbonate until the effervescence ceases. Make up the volume to 100 mL and centrifuge at 10,000 rpm 10 min. Take 0.2 ml of the sample solution in two separate test tubes. Make up the volume in each tube to 1 mL with water. Set a blank with 1 mL of water. Add 1 mL of phenol solution to each tube Add 5 mL of 96% sulphuric acid to each tube and shake well. After 10 min shake the content in the tubes and place in water bath at 25-30 °C for 20 min. Read the color at 490 nm.

Calculate the amount of total carbohydrate present in the sample solution using the standard graph prepared with known conc. of glucose (mg/ml)

Absorbance corresponds to 0.1mL of the test = ‘x’ mg of glucose

100 mL of the sample solution contains = ’x’ / 0.1 × 100 mg glucose.

= % of carbohydrates present.

Determination of Alkaloids: A total of 100 mL of 20% acetic acid was added to 2.5 g of leaf powder in a 150 mL beaker and covered to stand for 4 h. This mixture containing solution was filtered and the volume was reduced to one quarter using a water bath. To this sample, concentrated ammonium hydroxide was added drop-wise until the precipitate was complete. The whole solution was allowed to settle, and the precipitate was collected by filtration and weighed [8]. The percentage of total alkaloid content was calculated as:

Percentage of total alkaloids (%) = Weight of residue × 100 / Weight of sample taken.

Total Phenolic Content: The total phenolic contents of different solvent extracts of leaf of *Brassica juncea* var. rugosa were determined according to the method described by Malik and Singh [13]. 1 mg/ml of the extracts were taken made up to a volume of 1 ml with distilled water. Then 0.5 ml Folin- Ciocalteau reagent (1:1 with water) and 2 ml Na_2CO_3 (20%) were added. The mixture was shaken well and incubated in dark condition for 40 min. The test solutions were warmed for 1 min, cooled and absorbance was measured at 650 nm against the reagent used as a blank. A standard calibration plot was generated at 650 nm using known concentrations of catechol (mg/ml). The concentrations of phenolic content in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent /g of sample.

Total Flavonoids Contents: The aluminum chloride method (Willet 2002) was used for the determination of the total flavonoid content of the sample extracts [14]. 100 µl (1 mg/ml) of different solvent extracts were taken and made up the volume to 3 ml with ethanol. Then 0.1ml $AlCl_3$ (10%), 0.1 ml Na-K acetate, and 2 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance at 415 nm was recorded after 40 min of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent/g of sample.

Estimation of Tannins Content: Total tannins were determined by Folin-Denis method (Polshettiwar *et al*., 2007) [15]. 200 µl of the extract made up to 1 ml with distilled water and 0.5 ml of FC reagent and 2.5 ml of 20% sodium carbonate was added. The mixture was incubated for 40 min and absorbance was measured at 725 nm. The std. graph was prepared using tannic acid (µg/µl).

***In-vitro* Antioxidant Activity:**

DPPH Radical Scavenging Activity: Antioxidant as DPPH radical-scavenging activity was performed by the method described by Katerere and Eloff (2005), with some modification [7]. For each determination, the stock solution (1 mg/ml) was diluted to a dilution series (50 µg-150 µg-250 µg/ml) with respective solvents (v/v). An aliquot of each dilution (3 mL) was mixed with a methanolic solution of DPPH (1ml mL). The mixtures were shaken vigorously and incubated at 37 °C in the dark for 30 min. At the same time, a control containing 3 ml (v/v) methanol and 1 mL methanolic solution of DPPH was added. The absorbance was measured at 517 nm against methanol as a blank.

The percentage of DPPH scavenging was calculated as follows:

DPPH radical scavenging activity (%) = [(Abs control- Abs sample) / Abs control] × 100

Peroxidase Activity: Guaiacol peroxidase activity was measured according to Fielding and Hall (1978) [16]. This method is based on monitoring the GPOX scavenging activity by using guaiacol as a hydrogen donor. The reaction mixture contained 30 µl H_2O_2 in 3 ml phosphate buffer (pH 7), 50 µl guaiacol, and the enzyme extract in a total volume of. GPOX activity was estimated by the increase in the absorbance of tetra-guaiacol at 470 nm and was expressed as micromole of guaiacol oxidized per min at 25 ºC.

RESULTS:

Preliminary Phytochemical Analysis: Preliminary phytochemical analysis from the different solvent extracts of the leaf of *Brassica juncea* var. rugosa shows the presence of alkaloids, flavonoids, tannins, phenols, saponins, steroids, terpenoids, proteins, and carbohydrates. Alkaloids were found to be absent in chloroform extracts and petroleum ether and aqueous extracts show highest alkaloids presence. Same as alkaloids, flavonoids

were also found to be absent in the chloroform extracts and showed highest with the methanolic extracts. Most of the plant phytochemicals were found to be absent in the chloroform extract.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF DIFFERENT SOLVENT EXTRACTS OF LEAF OF *BRASSICA JUNCEA* VAR. *RUGOSA*

Phytochemical test	Petroleum ether	Ethyl acetate	Chloroform	Methanol	Water
Alkaloids	++	+	-	+	++
Flavonoids	+	+	-	+++	+
Phenols	-	+	++	+++	+++
Saponins	++	++	++	++	+
Steroids	++	++	++	++	+
Tannins	-	+	+	+	+
Terpenoids	+++	++	-	++	+
Carbohydrates	++	++	++	+	++
Proteins	+	+	-	+++	+

+++ highly present, ++ moderately present, +least present, - absence

Determination of Total Proteins and Carbohydrates: Determination of total proteins present in the fresh leaf sample of *Brassica juncea* var. rugosa was done by Bradford methods with slight modifications using BSA as standard [12]. Amount of total proteins was found to be 2.2 mg/g. **Table 2**. Total carbohydrates were estimated by Phenol-Sulphuric method with glucose as standard [12]. The amount of carbohydrates present in the fresh leaf sample of *Brassica juncea* var. *rugosa* was found to be 16.66 mg/g **Table 2**.

TABLE 2: QUANTIFICATION OF PROTEINS AND CARBOHYDRATES OF THE FRESH LEAF SAMPLE

Sample	Carbohydrates (mg/g)	Proteins (mg/g)
Brassica juncea var. *rugosa*	16.66 ± 2.8	2.2 ± 0.6

Experiments were performed in triplicates and values were represented as mean ± SD

Total Alkaloids Determination: The gravimetric determination of total alkaloids present in the leaf of *Brassica juncea* var. rugosa was found to be 88 mg/1g, and total alkaloid percentage was 8.8% as shown in **Table 3**.

TABLE 3: TOTAL ALKALOIDS CONTENT IN THE DRY SAMPLE OF THE LEAF OF *BRASSICA JUNCEA* VAR. RUGOSA

S. no.	Sample	Wt. of residue (88mg/1g)	Total alkaloids %, Wt. of residue × 100/wt. of sample
1	*Brassica juncea* var. rugosa	88 ± 7.73	8.8%

Experiments were performed in triplicates, and the data were expressed in mean ± SD.

Extraction Yield: The percentage yield of different crude extracts (*i.e.* petroleum ether / chloroform / ethyl acetate / methanol /water) of leaf sample of *Brassica juncea* var. rugosa were found to be highest in aqueous extract of 15%, followed by methanolic extract of 12%, which is then followed by ethyl acetate extracts of 3.60%, followed by petroleum ether extracts of 2.88% and the lowest yield was obtained from chloroform of 0.82% **Table 4**.

TABLE 4: PERCENTAGE YIELD OF DIFFERENT EXTRACTS OBTAINED FROM SOXHLET EXTRACTION OF LEAF OF *BRASSICA JUNCEA* VAR. RUGOSA

Sample	Percentage yield %
PE	2.88%
CH	0.82%
EA	3.60%
ME	12%
WA	15%

PE- Petroleum ether, CH-chloroform, EA-Ethyl acetate, ME-Methanol, WA- water

Total Phenolics Content: Total phenolics content were estimated by Malik and Singh Method with catechol as standard [13]. Among the different crude extracts, the methanolic extract showed highest phenolic content of 6.52 mg/g, followed by aqueous extracts (5.15 mg/g), which is then followed by ethyl acetate extracts (1.27 mg/g) and the one with petroleum ether and chloroform extracts showed almost same content which is 0.57 mg/g and 0.53 mg/g respectively **Table 5**.

Total Flavonoids Content: Total flavonoid content was estimated by the aluminum chloride method (Willet 2002) with quercetin as standard [14]. Total flavonoids content were found to be highest in petroleum ether extracts of 6.12 mg/g, followed by ethyl acetate 2.41 mg/g, followed by aqueous extract 1.53 mg/g, which is then followed by methanol extract of 1.25 mg/g and the least flavonoids content was found in chloroform extract 0.04 mg/g **Table 6**.

TABLE 5: TOTAL PHENOLIC CONTENT OF DIFFERENT SOLVENT CRUDE EXTRACTS OF LEAF OF *BRASSICA JUNCEA* VAR. *RUGOSA*

Extracts	Total phenolic contents (mg/g)
PE	0.57 ± 0.01
CH	0.53 ± 0.005
EA	1.27 ± 0.02
ME	6.52 ± 0.11
WA	5.15 ± 0.19

PE- Petroleum ether, CH-chloroform, EA-Ethyl acetate, ME-Methanol, WA- water. Experiments were performed in triplicates and the values were expressed as mean ± SD.

TABLE 6: TOTAL FLAVONOIDS CONTENT IN DIFFERENT CRUDE EXTRACTS OF LEAF OF *BRASSICA JUNCEA* VAR. RUGOSA

Extracts	Total flavonoids contents (mg/g)
PE	6.12 ± 0.06
CH	0.04 ± 0.004
EA	2.41 ± 0.06
ME	1.25 ± 0.04
WA	1.53 ± 0.03

PE- Petroleum ether, CH-chloroform, EA-Ethyl acetate, ME-Methanol, WA- water. Experiments were performed in triplicates and the values were expressed in mean ± SD.

Total Tannin Content: Quantitative estimation of total tannin content was done by Folin-Denis method (Polshettiwar *et al*., 2007) with tannic acid as standard [15]. Total tannin content was found to be highest in water extract 3.65 mg/g, followed by methanol extracts 2.64 mg/g, followed by petroleum ether 0.52 mg/g, ethyl acetate 0.50 mg/g, and chloroform 0.48 mg/g **Table 7**.

TABLE 7: TOTAL TANNINS CONTENT IN DIFFERENT CRUDE EXTRACTS OF LEAF OF *BRASSICA JUNCEA* VAR. RUGOSA

Extracts	Total tannins content (mg/g)
PE	0.52 ± 0.01
CH	0.48 ± 0.006
EA	0.50 ± 0.001
ME	2.64 ± 0.05
WA	3.65 ± 0.05

PE- Petroleum ether, CH-chloroform, EA-Ethyl acetate, ME-Methanol, WA- water. Experiments were performed in triplicates and the data were expressed in mean ± SD.

Antioxidant Activity using DPPH Assay: The free radical-scavenging activity of the *Brassica juncea* var. rugosa extracts was tested through DPPH method Akter *et al*., (2010) and results were compared with ascorbic acid [7]. At the concentration of 50 µg/ml, of the different extracts which was tested, methanol extract exhibited the highest antioxidant activity of (79.3%), followed by ethyl acetate (41.16%), petroleum ether (40.33%) and chloroform extract (10.3%), respectively at the same concentration **Fig. 1**. At concentration of 150 µg/ml, highest antioxidant activity was shown by methanol extracts (86.353%), ethyl acetate 52.3%, petroleum ether 50.2%, water 34.07% and chloroform extract 10.12% respectively **Fig. 1**. At concentration of 250 µg/ml, methanol extract showed 90.32%, ethyl acetate 56.133%, petroleum ether 55%, water 35.003%, chloroform 13.89% respectively **Table 8**.

TABLE 8: DPPH RADICAL SCAVENGING ACTIVITY OF DIFFERENT EXTRACTS OF VARIOUS CONC. OF LEAF OF *BRASSICA JUNCEA* VAR. RUGOSA

Conc. (µg/ml)	PE	CH	EA	ME	WA	AA
50	40.33%	10.3%	41.16%	79.3%	34.15%	97.2333%
150	50.2%	10.12%	52.3%	86.353%	34.0766%	98.8166%
250	55%	13.89%	56.133%	90.32%	35.003%	99.25633%

Experimental results are means ± SD of three parallel measurements.

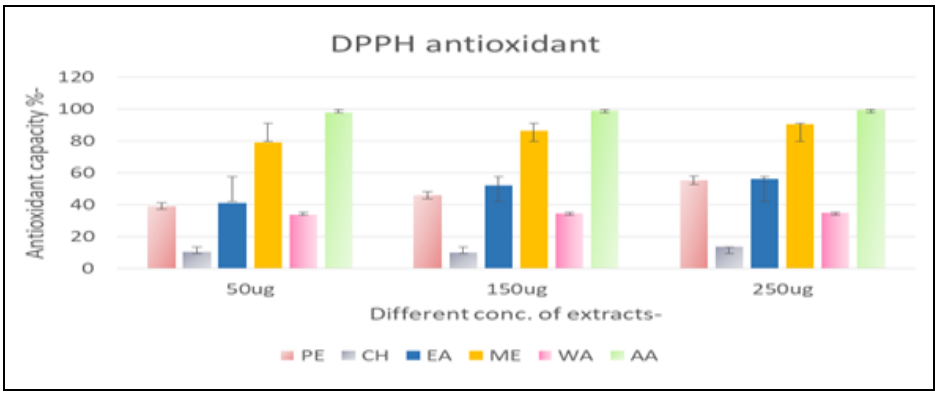

FIG. 1: PERCENTAGE OF DPPH ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF VARIOUS CONC. OF LEAF OF *BRASSICA JUNCEA* VAR. RUGOSA WITH REFERENCE TO ASCORBIC ACID. Experiments were performed in triplicates and the data were expressed in mean ± SD. PE- Petroleum ether, CH-chloroform, EA-Ethyl acetate, ME-Methanol, WA-water, AA- Ascorbic acid.

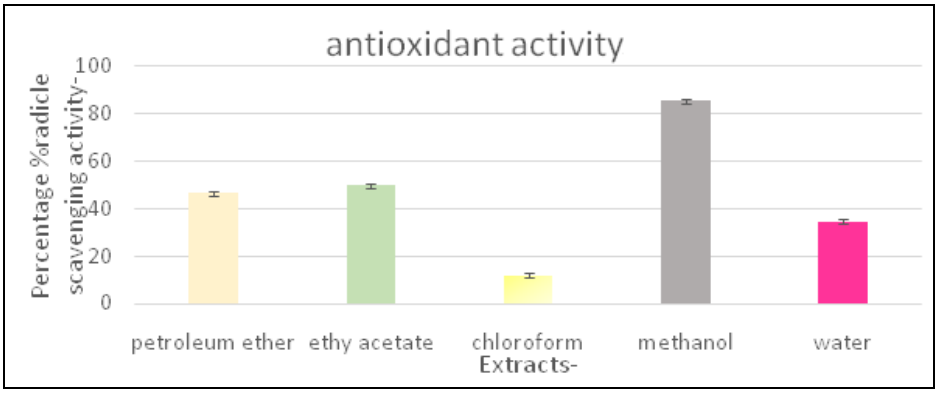

FIG. 2: FREE RADICAL-SCAVENGING ACTIVITY OF THE EXTRACTS FROM *BRASSICA JUNCEA* VAR. RUGOSA LEAVES AT 250 µg/ml CONCENTRATION BY DPPH METHOD. EACH SAMPLE WAS ASSAYED IN TRIPLICATE FOR EACH CONCENTRATION. Experimental results are means ± SD of three parallel measurements.

Peroxidase Activity: Guaiacol peroxidase activity was measured according to Fielding and Hall (1978) [15]. At 50 µl of the plant, leaf extracts peroxidase activity was found to be 22.512, and the time is taken for the reaction was 22.21 sec **Table 9**.

TABLE 9: PEROXIDASE ACTIVITY IN 1 g LEAF EXTRACTS OF *BRASSICA JUNCEA* VAR. RUGOSA

Sample	Concentrations of extract taken	Time taken 0.05 -0.1 Δt	EA = 500/Δt
Brassica juncea var. rugosa	50µl (in 1g leaf sample)	22.21 sec	22.512

DISCUSSION: Preliminary qualitative phytochemical analysis of the different successive crude extracts of leaf of *Brassica juncea* var. rugosa revealed the presence of alkaloids, flavonoids, proteins, carbohydrates, phenols, saponins, steroids, tannins, terpenoids. These secondary metabolites are reported to have many biological and therapeutic properties, so this species is expected to have many medicinal uses [10, 11]. The phytochemical properties and antioxidant potential of Brassica plants make them the preferred candidates for nutritional and pharmaceutical applications. Due to the presence of these compounds, Brassica plants show biological activities against various diseases and have been found to be effective in treating various diseases in human [7-21].

The plant also shows presence of high carbohydrate and protein. Since carbohydrates and proteins are nutritional compounds presence of these showed that the plant is very good for consumption as it has a high nutritional quality. These days acquiring a plant-based diet has become very popular, for moral reasons, yet in addition to ecological supportability, and wellbeing reasons [12]. Proteins are the building blocks of our body. They are used to build and repair tissues. Plant-based proteins are believed to have lower fat and cholesterol and provide fiber and other health-promoting nutrients [22].

The main source of energy for our body is Carbohydrates, and they have fiber. Eating food with fibre can prevent stomach or intestinal problems, such as constipation and additionally helps in bringing down glucose level and cholesterol level [23, 24]. Including this plant in one's balance diet will surely benefit human health in many ways.

The leaves of *Brassica juncea* var. rugosa showed 8.8% of alkaloids. Alkaloids in plants help in protection against destruction by certain pathogens. The therapeutic properties of alkaloids are very differing. Morphine which is a powerful narcotic used for the relief of pain. Codeine, which is found in the opium poppy, is an excellent analgesic that is relatively non-addictive [25-27]. Since, the plant showed the presence of high alkaloids, it can be concluded that this particular *Brassica juncea* var. rugosa also has high medicinal properties which can be further studied as a potential candidate for production of medicines.

The total phenolic content of the extracts from leaves of *B. juncea* var. rugosa was determined by Folin-Coicalteu method [12,] and the results are expressed as equivalents of catechol. Among the five extracts, methanol extract had the highest amount of phenolic compounds followed by

chloroform, ethyl acetate, water, and petroleum ether. These phenolic compounds are one of the main secondary metabolites present in the plant.

They have been reported to have many biological effects on the plants as well as other living organisms. They help in the growth and reproduction of plants and are produced as a response for defense against pathogens [28-30]. Presence of these phenolic contents can be related to the antioxidant properties. Present-day researchers are now reliably prescribing to take Brassicaceae vegetables specifically, for helping patients experiencing, or inclined to, diabetes and related psychological wellness conditions. However, not much effort has been given in designing and developing a proper well standardized pharmacological products, especially suited for such purposes [31]. So, with further study and analysis this plant can be used as modern traditional ayurvedic medicines.

For flavonoids, among the different extracts petroleum ether shows highest total flavonoids content followed by ethyl acetate followed by water, methanol, and chloroform. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties [32-34]. For tannins, which is calculated by taking tannic acids as STD. The different extracts give different concentration level of tannins. Among the 5 solvents, extracts chloroform shows the highest followed by water, methanol, petroleum ether, and ethyl acetate. Tannins are polyphenolic compounds that are present in various parts of plants. The consumption of tannins rich food will offer a lot of remedial and beneficial effects on human being. The tannin can also be used as drugs for healing the burning injury and for blood clothing [35].

For *in-vitro* antioxidant activity, the extracts exhibited a concentration-dependent antiradical activity by inhibiting DPPH radical. Of the different extracts, methanol extract exhibited the highest antioxidant activity of 90.32% at 250 µg/ml concentration, followed by ethyl acetate (56.133%), petroleum ether 55%, water 35% and then chloroform extract 13.89%, respectively at the same concentration. Antioxidants are the compounds which prevent the oxidation of the biomolecules by reducing the oxidizing agents. These compounds have the ability to scavenge the free radicals and they can minimize the oxidative stress which is produced during the redox reactions and helps in preventing the oxidative damage to plants and other living organisms [7, 8]. In humans antioxidants help in the prevention of coronary and vascular diseases and tumor formation [36].

Since the present plant *Brassica juncea* var. rugosa shows high antioxidant properties the plant can be consumed as supplements for the prevention of cancer, vascular disease, and anti-aging. The free radical scavenging potential of plant solvent extracts can be interrelated to the present high phenolic compounds in more polar solvents. More polar solvents can often extract antioxidant compounds in higher quantities [8, 29]. Antioxidant properties can also be related to the presence of polyphenol compounds. These polyphenol compounds are believed to help in the higher antioxidant properties [7]. In the case of the present study high polarity solvent, *i.e.* methanol showed highest phenolic contents and highest properties as compared to other polar solvent and nonpolar solvents like petroleum ether, chloroform, and ethyl acetate.

So the plant can be further studied and used as a potential candidate for the manufacturing of drugs which are useful in chemotherapy. The plant also showed good percentage of peroxidase activity so intake of this plant will help in the activity of scavenging Reactive oxygen species as peroxidase are the compounds which help in conversion of toxic H_2O_2 into water making it nontoxic to plants and human [17]. It can serve as a potential tool in biocatalysis.

CONCLUSION: The plant *Brassica juncea* var. rugosa showed the presence of many phytochemical compounds. These Phytochemical compounds are also known as plant secondary metabolites and are reported to have many biological and medicinal properties, so this species is expected to have many therapeutic uses and can be further studied for the production of pharmaceutical drugs. The plant showed very high nutritional contents. It showed presence of high proteins, carbohydrates. With the presence of all these plant *Brassica juncea* var. rugosa can be

proved as a healthy plant for consumptions and it can be added to one's balanced diet and can be taken as a food dietary supplements which will improve our body health in many ways.

The plant also showed high antioxidant properties. Antioxidants help in the prevention of cellular damage, by neutralizing the free radicals. So, this plant can also be used as a potential product for the manufacturing of drugs which are useful in chemotherapy. The main purpose of this present study is also to find out the new natural antioxidant sources from *Brassica juncea* var. rugosa. With the presence of high peroxidase activity in this plant, we can say that this particular *Brassica* plant can be utilized for the generation of industrially accessible plant peroxidase which will help the plants and humans in various ways.

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CONFLICTS OF INTEREST: The authors hereby declare no conflict of interest in the publication of this paper.

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