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Ethno pharmacologically active Components of *Brassica Juncea* (Brown Mustard) Seeds

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Abstract
Brassica juncea (brown mustard) seed is an economically important plant that has been well-known in many countries for centuries for its medicinal and nutritive values. Mustard seeds are used in various traditional remedies to stimulate appetite and as a laxative, expectorant and antiseptic agent for treatment of various gastrointestinal, respiratory and skin diseases. The aim of the present study was to determine the ethno pharmacologically active components of brown mustard seeds by performing qualitative and quantitative phytochemical, proximate and mineral analysis using the standard method of Association of Analytical Chemist (AOAC) and Atomic Absorption Spectrophotometric (AAS) method. The results show that qualitative phytochemicals present are: tannin, terpenoid, saponin, flavonoid, alkaloid, phenols and cardiac glycoside. Quantitatively, tannin content was (7.75 ± 0.06%), Terpenoid (5.40±0.02), Saponin (4.25 ± 0.01%), alkaloids (2.25 ± 0.01%), Flavonoid (2.48 ± 0.02%) and Phenols (0.12± 0.02). Proximate results show ash (0.23%), moisture (1.26%), crude fibre (1.80%), crude protein (2.58%) crude lipid (3.85%), Dry matter (98.74 ± 0.03) and Nitrogen free element (91.87±0.57). Mineral contents shows the presences of Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sodium (Na), Iron (Fe), Zinc (Zn), Manganese (Mn) and Copper (Cu). This available preclinical information strongly suggests that *B. juncea* could be a sustainable source of affordable drugs which is potentially a good source for obtaining pharmacologically standardized phytotherapeutics.

Keywords: Ethnopharmacology, Mustard seed, Secondary metabolites, drugs

1. Introduction
Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Researchers have great interest in screening of medicinal plants for therapeutics. The active principles of many drugs found in plants are secondary metabolites [1]. In folk medicine, medicinal herbs and plant products were used in treating a wide range of infections and other diseases [2, 3]. Today, a great number of different medicinal plant products are available in the market (including cosmetics and pharmaceuticals), which contains biologically active substances. In recent years, there has been a gradual revival of interest in the use of medicinal and aromatic plants in developed as well as in developing countries, because plant-derived drugs have reported to be safe and without side-effects.
Brassica juncea is an economically important plant that has been well known in many countries for centuries for its medicinal and nutritive values. Various parts of the plants are edible and used in a range of folk medicines and spices. The seeds are usually about 1 to 2 mm in diameter. The mustard plant belongs to the *Cruciferae (Brassicaceae*) family. Mustard used in food is often a mixture of seeds from two or more species of *Brassicaceae*, for example *Sinapis alba L*. (white or yellow mustard), *Brassica nigra* (black mustard) and *Brassica juncea L*. (Brown or oriental mustard). Mustard plant at different types have been widely cultivated and used as spice, medicine and a source of edible oil since ancients times [4]. Mustard is also used in various traditional remedies to stimulate appetite and as a laxative, expectorant and antiseptic agent for treatment of various gastrointestinal, respiratory and skin diseases [5].
There are various polyherbal formulations of mustard discussed in Ayurveda medicine. A decoction of *Moringa oleifera* root (1 in 20) with the addition of bruised mustard seed is useful in doses of 1-2 ounces in ascites due to liver and spleen diseases [6]. According to Ayurveda medicine, mustard preparations are mild laxatives, diuretics, and liver-bile stimulators [7] and have been also documented to purge the toxins out of the body [8]. The leaves and seeds of these plants are edible and diverse medicinal uses of seeds are also well known in other countries. In China, mustard seed is a folk remedy for arthritis, foot ache, lumbago and rheumatism.
It is also used in the treatment of tumors; leaves are used in soups for bladder infections, inflammation or haemorrhage. In Korea, the seeds are used for abscesses, colds, and lumbago, rheumatism and stomach disorders. The seeds are also reported to be hypoglycemic [9], antioxidant [10], anti-diabetic [11], hyperglycemic [12], anxiolytic [13], and goiterogenic [14].
This research was aimed at determining the ethno pharmacologically active components of *Brassica juncea* (Brown mustard) seeds.

2. Materials and Methods
2.1. Collection of Plant material
*Brassica juncea (*Brown mustard seed) was bought from the market in Delta state. The seeds were pulverized and stored in an airtight container for laboratory analysis.

2.2. Methods
2.2.1 Qualitative Phytochemical Screening
Phytochemical screening of the extracts was carried out by a

Procedure that was based on those earlier reports by [15].

2.2.2.1. Test for saponins
0.5 g of the plant extract was added to 5 ml of distilled water in a test tube. The solution was vortexed and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil, vortexed and formulation of an emulsion was observed.

2.2.2.2. Test for terpenoids
Plant extract of 0.5 g was dissolved in 1 ml of chloroform and 1 ml acetic anhydride added, with 2 ml of concentrated H_2SO_4. Formation of reddish violet colour was observed.

2.2.2.3. Test for tannins
Plant extract of 0.5 g was boiled in 10 ml of water in a test tube and filtered. A few drops of 0.1 % ferric chloride were added and the solution observed for brownish green or a blue-black colouration.

2.2.2.4. Test for cardiac glycosides (keller-killiani test)
0.5 g of plant extract dissolved in 5 ml water was added 2 ml of glacial acetic acid solution containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

2.2.2.5. Test for flavonoids
5 ml of dilute ammonia was added to a portion of an aqueous filtrate of the extract. Then, 1 ml concentrated sulphuric acid was added. A yellow colouration indicated the presence of flavonoids.

2.2.2.6. Test for alkaloids
Plant extract was dissolved in dilute HCl and filtered. Filtrates were treated with Mayer`s reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

2.2.3 Quantitative Phytochemical Analysis
2.2.3.1 Total Tannins Content Determination
Total tannins were determined by slightly modified Folin and Ciocalteu method. 0.5 ml of the plant extract was added with 3.8 ml of distilled water and 0.25 ml of Folin Phenol reagent, 0.5 ml of 35% sodium carbonate solution was added. The absorbance was measured at 725 nm. Tannic acid dilutions (0 to 0.5mg/ml) were used as standard solutions. The results of tannins are expressed in terms of tannic acid in mg/ml of extract.

2.2.3.2 Total Phenol Content Determination
Total phenols were determined by slightly modified Folin and Ciocalteu method. 200µl of the plant extract, 800 µl of Folin Ciocalteu reagent mixture and added 2 ml of 7.5% sodium carbonate. The total content was diluted to 7 volumes with distilled water and finally kept the tubes for 2 hrs incubation in dark. The absorbance was measured at 765 nm. Gallic acid dilutions were used as standard solutions. The results of phenols were expressed in terms of Gallic acid in mg/ml of extract.

2.2.3.3 Total Alkaloid Content Determination
40 ml of 10% acetic acid in ethanol was added to 1 g of powdered sample, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to get 1/4th of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and collected precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

2.2.3.4 Total Flavonoid Content Determination
Total flavonoids content of samples was determined by following the Aluminum chloride method. Plant concentrate was mixed with distilled H_2O and $NaNO_2$ solution. After 6 min, $AlCl_3$ solution was added and enabled to stand for 6 min, NaOH solution was added to the mixture. Immediately distilled H_2O was added to bring to the final volume and then the mixture was extensively mixed and enabled to stand for another 15 min. Optical density of the mixture was recorded at 510 nm. Rutin was used as a standard compound for the evaluation of total flavonoid. The total flavonoids were calculated using the standard curve, and expressed as rutin equivalent in mg/g of extracts.

2.2.3.5 Total Saponin Content Determination
Plant extract were dissolved in 80 % methanol, 2 ml of Vanilin in ethanol was added, mixed well and the 2 ml of 72 % sulphuric acid solution was added, mixed well and heated on a water bath at 600c for 10 min, absorbance was measured at 544 nm against reagent blank. Diosgenin is used as a standard material and compared the assay with Diosgenin equivalents.

2.2.3.6 Total Terpenoid Content Determination
Plant extract (1 g) was marcarated with 50 ml of ethanol and filtered. To the filtrate (2.5 ml), 2.5 ml of 5 % aqueous phosphomolybdic acid solution was added and 2.5 ml of concentrated H_2SO_4 was gradually added and mixed. The mixture was left to stand for 30 min and then made up to 12.5 ml with ethanol. The absorbance was taken at 700 nm.

2.2.4 Methods for Proximate Analysis
The dry matter, moisture, ash, crude fat, crude protein (nitrogen x 6.25) and crude fibre contents were analyzed in powdered brown mustard seed using the standard methods of the Association of Official Analytical Chemists [16] while Dry Matter and Nitrogen Free Element contents was calculated based on the net difference between the other nutrients and the total percentage composition.

2.2.4.1 Estimation of ash
About 2g of the sample was weighed and taken in a vitreosil basin. The basin was heated in a low flame at the beginning till no fumes were given off by the charred mass. It was broken by a glass rod carefully and burnt in a muffle furnace at 550- 600°C for 4-5 hrs. The muffle was allowed to cool to 150°C. The basin was then cooled in a desiccator and the ash content was then weighed. The total ash was calculated as follows:

$$\%\ of\ total\ ash = \frac{weight\ of\ the\ ash\ \times 100}{weight\ of\ the\ sample}$$

2.2.4.2 Estimation of moisture content
Fresh sample materials were taken in a flat bottom dish and kept overnight in a hot air oven at 100-110°C and weighed. The loss in weight was regarded as a measure of moisture content.

2.2.4.3 Estimation of crude protein (Micro-Kjeldahl Method)
Digestion: About 2gm of sample was taken in a Kjeldahl flask, 10gms of sodium sulphate and 0.5 gm of copper sulphate was added and mixed well. A few glass beads were added into the flask to prevent spurting while heating. Then 25 ml of concentrated H_2SO_4 was added and then heated for 15-20 mins in inclined position. The solution was boiled until a greenish colour was obtained. It was allowed to cool.

Distillation
About 100 ml of distilled water was added to the Kjeldahl flask, shaken properly and transferred it into a 250 ml volumetric flask. Then the final volume was made up to 250 ml by adding distilled water. In a conical flask, 10-15 ml of 2% Boric acid was taken and the flask was placed below the condenser of the distillation apparatus. Thereafter, 5 ml of aliquot was transferred to the Micro Kjeldahl steam distillation apparatus and added 1 drop of phenolphthalene and 10-15 ml 40% NaOH. The distillation was carried out at least for 5-10 mins until ammonia was free from aliquot. Titration: The distillation product was then titrated against N/10 H_2SO_4
Calculation is done as follows:

$$\%\ of\ Nitrogen = \frac{ml\ of\ \frac{N}{10} H_2SO_4\ used\ up\ \times 250 \times 0.0014 \times 100}{Volume\ of\ aliquot\ \times gm\ of\ the\ substance\ taken}$$

$$\%\ of\ crude\ protein = \%\ Nitrogen\ \times 6.25$$

2.2.4.4 Estimation of crude Lipid (Ether extract)
5gm of dry sample was weighed on a piece of glazed paper and transferred into an extraction thimble. The thimble was introduced into soxhlet extractor over a pad of cotton wool, so that top of the thimble is well above the top of the siphon. A clean dry flask was taken, weighed and was fitted with the extractor. Ether was poured along the side of the extractor until it begins to siphon off. Then another half-a siphonful of ether was added. The equipment thus assembled with the flask was placed on a water bath at 60-80°C and the extractor was connected with the condenser. Cool water circulation was started in the condenser and allowed the extraction for 8 hr. Then the thimble with the material was removed from the extractor. The apparatus was assembled again and heated on a water bath to recover all the ether from the receiver flask. The receiver flask was disconnected and dried it in a hot air oven at 100°C for 1 hr, cooled and weighed.

$$\%\ of\ Ether\ extract = \frac{(Wt.of\ oil\ flask\ with\ ether\ extract - Wt.of\ the\ oil\ flask) \times 100}{gm\ of\ the\ substance\ taken}$$

2.2.4.5 Determination of crude fibre
About 2 gm of moisture and fat free sample was weighed and transferred to the spout less one litre beaker. Thereafter, 200 ml 1.25% H_2SO_4 was added. The beaker was placed on hot plate and allowed to reflux for 30 mins, timed from onset of boiling. The content was shaken after every 5 min. The beaker was removed from the hot plate and filtered through a muslin cloth using suction. The residue was washed with hot water till it was free from acid. The material was transferred to the same beaker and added 200ml of 1.25% NaOH solution and refluxed for 30 mins. Again filtered and the residue was washed with hot water till it was free from alkali. The total residue was transferred to a crucible and placed in hot air oven, allowed to dry to a constant weight at 80-110°C and weighed. The residue was ignited in muffle furnace at 550-600°C for 2-3 hrs, cooled and weighed again. The loss of weight due to ignition was the weight of crude fiber.

$$\%\ of\ fiber = \frac{(Wt\ of\ the\ crucible\ with\ dry\ residue - Wt\ of\ crucible\ with\ ash)\ \times 100}{gm\ of\ the\ substance\ taken}$$

2.2.5 Procedure for Mineral analysis
For this study, 0.5 gm of powdered dried sample was taken in a crucible and converted to ash in the muffle furnace at 580°C for 3 hrs. After cooling in a desiccators 10 ml of concentrated Nitric acid, 4 ml of Perchloric acid and 1ml of Sulphuric acid was added and digestion at high temperature was carried out until the content became clear, then the tube was cooled and the solution was transferred quantitatively to 50 ml volumetric flask and the final volume was adjusted to 50 ml by adding distilled water. The solution was used for determination of Fe, Zn, Mg, Mn, Na, K and Cu through the atomic absorption spectrometry (AA203D). Calcium and Phosphorous estimation were done as per method described by Odangowei *et al* [17].

3. Results

3.1. Qualitative and Quantitative Phytochemical Results
Qualitative phytochemicals present are: tannin, terpenoid, saponin, flavonoid, alkaloid, phenols and cardiac glycoside. Quantitatively, tannin content was (7.75 ± 0.06%), Terpenoid (5.40±0.02), Saponin (4.25 ± 0.01%), alkaloids (2.25 ± 0.01%), Flavonoid (2.48 ± 0.02%) and Phenols (0.12± 0.02) (Table 1 & 2).

3.2. Proximate Results
Ash content (0.23%), moisture content (1.26%), crude fibre content (1.80%), crude protein content (2.58%) crude lipid content (3.85%), Dry matter (98.74 ± 0.03) and Nitrogen free element (91.87±0.57) (Table 3).

3.3. Mineral Results
Mineral results shows the presences of Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sodium (Na), Iron (Fe), Zinc (Zn), Manganese (Mn) and Copper (Cu) (Table 4).

4. Discussion
B. juncea are known to produce several classes of bioactive phytochemicals including glycosides, flavonoids, phenolic compounds, sterols, triterpene alcohols, glucosinolates (GLSs), proteins and carbohydrates. The available preclinical information on this easily cultivable and edible plant strongly suggests that it could be a sustainable source of affordable bioceuticals or drugs. The beneficial effects of *Brassica* vegetables on health improvement have been partly attributed to their complex mixture of phytochemicals possessing antioxidant activity [18].

Various classes of phytoconstituents from seeds of *B. juncea* were detected via qualitative screening and quantitative analysis. Table 1 shows the qualitative phytochemical components of brown mustard seed such as tannin, terpenoid, saponin, flavonoid, alkaloid, phenols and cardiac glycoside were present. Quantitatively, tannin content was highest (7.75 ± 0.06%) in the seeds of mustard. This bioactive substance quickens the healing of wounds and inflamed mucous membranes [19]. Tannins are known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues [20]. The value in this study is in agreement with the studies associated with other researchers in the same field [21, 22]. Terpenoid (5.40±0.02) and Saponin content (4.25 ± 0.01%) suggests the usefulness of the plant as a potential fertility agent. Alkaloids content (2.25 ± 0.01%) one of the most efficient therapeutically significant bioactive substances in plants. Pure isolated alkaloids and the synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bactericidal properties [23]. They are found in reducing stress and depression symptoms. Flavonoid content (2.48 ± 0.02%), inferred that the plant samples has the biological functions such as anti-oxidation, and protection against allergies, inflammation, free radical, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumour [24,19]. Phenols (0.12± 0.02) and Cardiac glycosides are important class of naturally occurring drugs whose actions helps in the treatment of congestive heart failure [25]. Considering the health benefits, establishing the therapeutic potential of the seeds, phenolics and flavonoids are critical active components.

The proximate compositions of the seeds are shown in Table 3. The seeds have a lower percentages of ash (0.23%), moisture (1.26%) and crude fibre (1.80%) while higher percentages of crude protein (2.58%) and crude lipid (3.85%). The data were in good agreement with [26], [27] and [28]. The present study showed that with respect to the crude protein and crude lipid, the seed could be added to low protein nutritional foods. Also from the result, the observed low moisture content in the mustard seed sample is an indication that the activity of microorganisms would be reduced and thereby increases the shelf life of the seed powder. The seed sample had higher lipid content 3.85%, this high crude lipid in the seed sample suggested that mustard seed is a good source of quality vegetable oil for both domestic and industrial purposes.

The result of the mineral analysis is shown in Table 4. The result showed that mustard seeds had relatively higher value of Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg) and Sodium (Na) which are known to be macro element and are required in large quantity in human body. Also it is relatively lower in Iron (Fe), Zinc (Zn), Manganese (Mn) and Copper (Cu) which are micro element required in smaller quantity. The data are in good agreement with [29]. Also the elements, copper (Cu) and manganese (Mn) act as cofactors of antioxidant enzymes to protect the body from oxygen free radicals that are produced during oxidative stress [30].

5. Conclusion

The results obtained in this study shows that the seeds of *B. juncea* contain bioactive substances, tannin, terpenoid, saponin, flavonoid, alkaloid, phenols and cardiac glycoside. Also high levels of crude lipid, crude protein, crude fiber, ash and moisture, and macro and micro elements were present. As a rich source of secondary metabolites brown mustard seed can be a potential source of useful drugs which justifies the seeds use in various traditional remedies to stimulate appetite and as a laxative, expectorant and antiseptic agent for treatment of various gastrointestinal, respiratory and skin diseases. This available preclinical information on this easily cultivable and edible plant strongly suggests that it could be a sustainable source of affordable bioceuticals or drugs.

Table 1: Qualitative phytochemical Screening of Brown Mustard seed

Phytochemical components	Brown mustard seed
Alkaloid	+
Tannin	+
Saponin	+
Flavonoid	++
Cardiac Glycoside	+
Terpenoid	++
Phenols	+

(Note: + present ++abundance)

Table 2: Quantitative phytochemical analysis of Brown mustard seed

Phytochemical components (%)	Brown mustard seed
Alkaloid	2.25±0.01
Tannin	7.75±0.06
Saponin	4.25±0.01
Flavonoid	2.48±0.02
Terpenoid	5.40±0.02
Phenols	0.12±0.02

Note: Values are mean ± SD of triplicate determination

Table 3: Proximate analysis of Brown mustard seed

Proximate Component (%)	Brown mustard seed
Moisture	1.26±0.03
Ash	0.23±0.01
Crude Protein	2.58±0.02
Crude Lipid	3.85±0.01
Crude Fibre	1.80±0.02
Dry Matter	98.74±0.03
Nitrogen Free Element	91.87±0.57

Note: Values are mean ± SD of triplicate determination

Table 4: Mineral Content of Brown mustard seed

Minerals (ppm)	Brown Mustard seed
Calcium (Ca)	48.72±2.00
Magnesium (Mg)	28.64±0.03
Sodium (Na)	1.48±0.03
Potassium (K)	62.54±0.03
Iron (Fe)	1.30±0.02
Manganese (Mn)	0.18±0.02
Copper (Cu)	0.02±0.01
Zinc (Zn)	0.40±0.02
Phosphorus (P)	78.50±0.02

Note: Values are mean ± SD of triplicate determination

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