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CHEMICAL AND BIOLOGICAL ASSAYS OF *BRASSICA RAPA* SUBSP. *CHINENSIS* (L.) HANELT

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Abstract

Bangladesh is in the tropical zone blessed with many plants and people of this country are dependent on them to a greater extent for foods and medicines. *Brassica* is a broad genus and encompasses a lot of herbs. Among these, *Brassica rapa* subsp. *chinensis* (L.) Hanelt (Family-Brassicaceae) is famous in Bangladesh as vegetables. In order to explore the healing abilities of *B. rapa* subsp. *chinensis*, different chemical and biological assays have been conducted. Initially, the sun-dried powdered herb was extracted well with methanol and then partitioned with petroleum ether, carbon tetrachloride, chloroform and aqueous soluble fractions. All these extractives were subjected to brine shrimp lethality, free radical scavenging, phenolic content determination and antimicrobial assays. *B. rapa* subsp. *chinensis* exhibited various responses in the conducted assays depending on the extractives. These studies revealed the facts to consider this plant with medicinal qualities in addition to its general identity as vegetables.

Natural products have earned an admirable place in drug discovery. The lead molecules obtained from natural sources are frequently used to produce many derivatives as drug candidates. Screening of plants focusing on bioactivities is essential for exploring the desired drug molecules (Benites *et al.* 2012, Christen and Cuendet 2012).

Plants are the natural reservoir of secondary metabolites and brine shrimp lethality bioassay is a popular bench-top tool to screen out these bioactive metabolites (Meyer *et al.* 1982, Rahman *et al.* 2008a). Free radicals are considered as notorious oxidizing elements in our body to promote aging, lipid peroxidation, inflammation and other pathologies. Plants are good sources of antioxidants and proper screening might find out the active antioxidants (Chowdhury *et al.* 2010, Conner and Grisham 1996, Das 2012). Besides, secondary metabolites of plants are worthy to screen out for searching anti-infective agents. These are the life-saving elements available in market but drug resistance-issue promotes the need for new antimicrobials (Rahman *et al.* 2008b, Rodriguez-Noriega *et al.* 2014). Bangladesh has wider opportunity for screening out numerous plants for drug candidates.

Brassica rapa subsp. *chinensis* (L.) Hanelt (Bengali name: Bati shak) belongs to Brassicaceae. It has light green and thin leaves. Base of the leaf petioles contains white-flesh roots. The flowers of this herb remain as cluster on the top of the raceme. This plant is popular for providing nutrients, vitamins and minerals (Dominguez-Perles *et al.* 2014, Siddiqui *et al.* 2014). Previous phytochemical studies of *Brassica* genus reported the isolation of some phenolics and organic acids (Fernandes *et al.* 2007, Mucha-Pelzer *et al.* 2010, Tenore *et al.* 2012).

As a part of our continuing studies on medicinal plants of Bangladesh (Begum *et al.* 2010, Islam *et al.* 2009, Rahman *et al.* 2011), *B. rapa* subsp. *chinensis* was screened for evaluating brine shrimp lethality, free radical scavenging, phenolic content and antimicrobial assays comprehensively.

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The whole plant of *B. rapa* subsp. *chinensis* was collected on June, 2013 from Gazipur, Bangladesh and a voucher specimen (DACB Accession No: 39457) has been deposited at Bangladesh National Herbarium, Mirpur, Dhaka for future reference. The collected plant was dried for a week and ground into a coarse powder. The powder was stored in an airtight container and kept in a cool, dark and dry place until experiment commenced. 500 g of the powdered materials was soaked in 1.5 litre of methanol at room temperature for 7 days. The extract was filtered through cotton plug and concentrated with a rotary evaporator. An aliquot (5 g) of the concentrated methanol extract was fractionated by the modified Kupchan method (VanWagenen *et al.* 1993) into petroleum ether, carbon tetrachloride, chloroform and aqueous soluble fractions. Subsequent evaporation of solvents yielded petroleum ether (PE, 0.70 g), carbon tetrachloride (CTC, 1.80 g), chloroform (CF 1.20 g) and aqueous (AQ 1.20 g) soluble materials, respectively.

In brine shrimp lethality bioassay, a simple zoological organism, *Artemia salina*, was used as a convenient monitor for the screening (Meyer *et al.* 1982, Rahman *et al.* 2008a). The eggs of the brine shrimp were hatched in artificial seawater (3.8% NaCl solution) for 48 hrs to mature shrimp called nauplii. The test sample of crude extract was prepared by dissolving them in DMSO (not more than 50 µl in 5 ml solution) plus sea water (3.8% NaCl in water) to attain concentrations of 12.5, 25, 50, 100, 200 and 400 µg/ml. A vial containing 50 µl DMSO diluted to 5 ml was used as a control. Standard vincristine sulphate was used as positive control. Then matured shrimps were applied to each of all experimental vials and control vial. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From the obtained data, the per cent of mortality of the brine shrimp nauplii was calculated. The median lethal concentration, LC_{50} was then determined using Probit analysis.

The free radical scavenging activity of the extract was determined by a chemical assay based on the scavenging activity of the stable 1,1 diphenyl-2-picrylhydrazyl (DPPH) free radical (Parvin *et al.* 2009). In brief, 2 ml of a methanol solution of the extract at different concentration were mixed with 3 ml of a DPPH methanol solution (20 µg/ml). Absorbance at 517 nm was determined after 20 min keeping in dark and the per cent inhibition was calculated from $[(A_o–A_1)/A_o] \times 100$, where A_o is the absorbance of the control and A_1 is the absorbance of the test sample. Here, BHT (*tert*-butyl-1-hydroxytoluene) and ascorbic acids were used as standards.

Total phenolic content of extracts were measured by a chemical assay through using Folin-Ciocalteu reagent as an oxidizing agent and gallic acid as a standard (Sikder *et al.* 2012). In this assay, 0.5 ml of plant extract (2 mg/ml) in water was mixed with 2.5 ml of Folin-Ciocalteu reagent (10 times diluted with water) and 2.0 ml of sodium carbonate (7.5 % w/v) solution. After 20 min of incubation at room temperature, the absorbance was measured at 760 nm using a UV-visible spectrophotometer. Total phenolics were quantified by calibration curve obtained from measuring the known concentrations of gallic acid (0 - 100 µg/ml) and were expressed as mg of GAE (gallic acid equivalent)/gm of the dried extract.

The disc diffusion method (Bauer *et al.* 1966, Rahman *et al.* 2008b) was used to test antimicrobial activity of the extractive against 13 bacteria, named *Bacillus cereus*, *B. megaterium*, *B. subtilis*, *Staphylococcus aureus*, *Sarcina lutea*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* Paratyphi, *S.* Typhi, *Shigella boydii*, *S. dysenteriae*, *Vibrio mimicus* and *V. parahemolyticus*. The bacterial strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka.

Three test samples were prepared for each of the assays. Values were expressed as mean ± standard deviation (SD).

Bangladesh is located in tropical zone facilitating the growth of numerous medicinal and dietary plants. People here are dependent on these green assets significantly but systematic

medicinal evaluations of these plants have not yet been done substantially. The present studies were designed to screen the free radical scavenging, phenolic content, antimicrobial and toxicity studies of *B. rapa* subsp. *chinensis* growing in Bangladesh. The evaluation has been done here with crude methanol extracts and its petroleum ether, carbon tetrachloride, chloroform and aqueous soluble fractions for all the assays.

The bioactivities of plants are generated from the secondary metabolites present in them. The brine shrimp lethality assay might be used to monitor potential bioactive and toxic metabolites of plant origin (Meyer *et al.* 1982, Rahman *et al.* 2008a). This study showed the presence of bioactive toxic natural compounds in the test samples of *B. rapa* subsp. *chinensis* (Table 1). The petroleum ether and chloroform soluble fractions exhibited higher level of toxicity with LC_{50} (concentration at which 50% of the test shrimps die) values 0.06 and 0.17 µg/ml, respectively.

Table 1. LC_{50} values of *B. rapa* subsp. *chinensis* and standard.

Test samples	LC_{50} (µg/ml) ± SD
Vincristine sulfate (standard)	0.45 ± 0.13
Methanol extract	5.22 ± 0.83
Petroleum ether soluble fraction	0.06 ± 0.02
Carbon tetrachloride soluble fraction	14.05 ± 1.13
Chloroform soluble fraction	0.17 ± 0. 30
Aqueous soluble fraction	73.00 ± 3.49

Excess free radicals and oxidants produce oxidative stress, which is a very injurious process that can affect the cell membranes and other structures such as proteins, lipids, lipoproteins and deoxyribonucleic acid. If not controlled, oxidative stress can induce a variety of chronic and degenerative diseases, aging process, trauma, inflammatory damages etc. (Conner and Grisham 1996, Das 2012, Srivastava *et al.* 2009). In the current study, *B. rapa* subsp. *chinensis* evidently scavenged the free radicals generated by DPPH to demonstrate its antioxidant activity (Fig. 1). The aqueous soluble fraction displayed the highest scavenging activity in this assay. The chloroform and carbon tetrachloride soluble materials were also very potent to display its scavenging action. In comparison to these, the petroleum ether fraction and crude methanolic extract demonstrated weaker antioxidant activity.

It is known that the phenolic compounds in plants serve as antioxidants. Higher the amount of phenolic compounds, higher will be the free radical scavenging activity. The current phenolic content determination study of *B. rapa* (Fig. 2) confirmed the presence of numerous antioxidant elements in this plant. The aqueous fraction showed the highest level of phenolics. Besides, chloroform and carbon tetrachloride soluble fractions were found to contain moderate amount of phenolic compounds. The petroleum ether soluble fraction and the crude methanol extract revealed relatively low amount of phenolics. Taken together, these assays showed that the free radical scavenging activity of the plant extractives is positively correlated with the amount of phenolics remaining in these extractives.

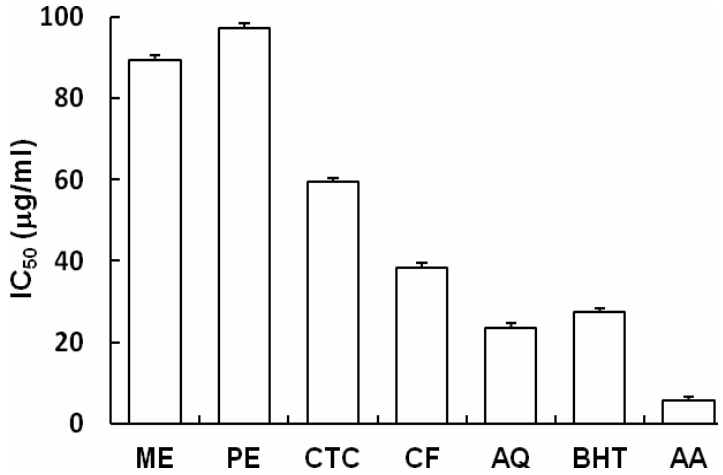

Fig. 1. The half maximal inhibitory concentration (IC_{50}) values of the standard and different partitionates of *B. rapa* subsp. *chinensis* Here, ME: crude methanolic extract; PE- petroleum ether soluble fraction of methanolic extract; CTC- carbon tetrachloride soluble fraction of methanolic extract; CF- chloroform soluble fraction of methanolic extract; AQ-aqueous fraction; BHT- *tert*-butyl-1-hydroxytoluene (standard); AA-ascorbic acid (standard).

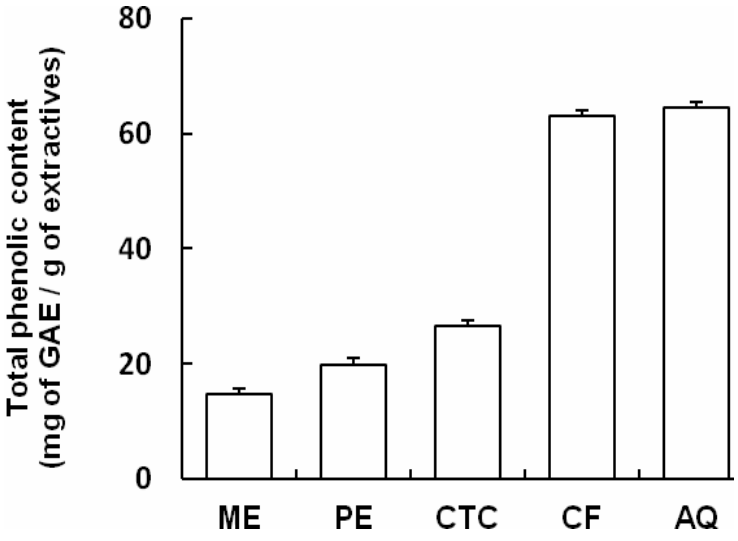

Fig. 2. Total phenolic content of *B. rapa* subsp. *chinensis* extractives. Here, GAE- gallic acid equivalent; ME: crude methanolic extract; PE- petroleum ether soluble fraction of methanolic extract; CTC- carbon tetrachloride soluble fraction of methanolic extract; CF- chloroform soluble fraction of methanolic extract; AQ-aqueous fraction.

The plant extractives were also subjected antimicrobial assay. Only the carbon tetrachloride and choloroform soluble fractions showed weak antimicrobial activities ranging from 7 to 10 mm of zone of inhibition at a dose 400 µg/disc (Table 2).

Table 2. Antimicrobial activity of *Brassica rapa* subsp. *Chinensis* and standard.

Bacteria	Diameter of zone of inhibition (mm) ± SD					
	ME	PE	CTC	CF	AQ	CIP
Gram positive bacteria						
Bacillus cereus	-	-	8.0 ± 1.0	9.0 ± 1.0	-	40.0 ± 3.0
B. megaterium	-	-	-	-	-	48.0 ± 0.0
B. subtilis	-	-	8.0 ± 2.0	8.0 ± 1.0	-	45.0 ± 1.0
Staphylococcus aureus	-	-	-	-	-	36.0 ± 3.0
Sarcina lutea	-	-	9.0 ± 2.0	10.0 ± 3.0	-	40.0 ± 4.0
Gram negative bacteria						
Escherichia coli	-	-	8.0 ± 1.0	-	-	40.0 ± 2.0
Pseudomonas aeruginosa	-	-	9.0 ± 1.0	9.0 ± 3.0	-	44.0 ± 1.0
Salmonella Paratyphi	-	-	9.0 ± 3.0	8.0 ± 1.0	-	35.0 ± 2.0
S. Typhi	-	-	7.0 ± 1.0	-	-	38.0 ± 1.0
Shigella boydii	-	-	-	-	-	38.0 ± 3.0
S. dysenteriae	-	-	-	7.0 ± 0.0		38.0 ± 2.0
Vibrio mimicus	-	-	-	-	-	37.0 ± 4.0
V. parahemolyticus	-	-	8.0 ± 2.0	9.0 ± 2.0	-	40.0 ± 1.0

A diameter less than 6 mm was considered inactive; ME: Crude methanol extract; PE: Petroleum ether soluble fraction; CTC: Carbon tetrachloride soluble fraction; CF: Chloroform soluble fraction; AQ: Aqueous fraction; CIP: Standard ciprofloxacin (30 µg/disc).

The plant *B. rapa* subsp. *chinensis* has many toxic secondary metabolites as evident from brine shrimp lethality bioassay. Besides, it is also rich in phenolics and antioxidants. Further phytochemical investigation is required to isolate the bioactive molecules from this plant.

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