 Phytochemical Screening of the Hydro Ethanolic Extract of *Brassica oleracea* Var. Italica Plant Extract

Shah M A1*, Himanshu1, Sarker M M R2,3, Banu Afreen4

1Department of Microbiology, OPJS University, Churu, Rajasthan. Email1: mashy786@gmail.com
2Department of Pharmacology, Faculty of Pharmacy, Lincoln University College, 47301 Petaling Jaya, Selangor Darul Ehsan, Malaysia.
3Department of Pharmacy, State University of Bangladesh, 77 Satmasjid Road, Dhammondi, Dhaka 1205, Bangladesh.
4Department of Microbiology, Faculty of Medicine, Lincoln University College, Malaysia.

Received 17th April, 17; Revised 23rd June, 17, Accepted 12th July, 17; Available Online 25th July, 2017

ABSTRACT
Objective: This study was designed elucidate the Phytochemicals of the widely-used plant *Brassica oleracea* var. Italica. Method: Hydroethanolic extracts of *Brassica oleracea* var. Italica plant extract was investigated. A small portion of the hydroethanolic extracts of *Brassica oleracea* var. Italica was subjected to the phytochemical test using Trease and Evans and Harbourne methods to test for the presence of alkaloids, tannins, reducing sugars, saponins, terpenoids, phenols, flavonoids and Anthraquionones. Result: The phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, quinines, cardiac glycosides, Xanthoproteins, glycosides, steroids, phenols, resins, carboxylic acid group in varying concentrations. The present study provides evidence that Hydro ethanolic extracts of *Brassica oleracea* var. Italica contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases. Conclusion: Thus, from the present study the plant leaf extracts of Brassica oleracea var. Italica showed an abundant production of Phytochemicals as secondary metabolites and they can be used in the pharmaceutical industries for producing a potent drug. The studies result of the above two plants gives a basis of its use in traditional medicine to manage ailments and disorders.

Keywords: Brassica oleracea var. Italica; ethanol extract; phytochemical analysis.

INTRODUCTION
World health organization (WHO) has estimated that approximately 80% of the world’s population from developing countries mainly relies on traditional medicines for the primary healthcare. Many of medicinal plants contain large amounts of antioxidants such as polyphenols which has an important role in the prevention and restriction of free radicals. Many of these phytochemicals have significant antioxidant potentials that are associated with lower occurrence and lower mortality rates of several human diseases (Anderson et al., 2001). Medicinal plants, either as an extract, pure compound or as a derivative, offer limitless opportunities for the discovery of new drugs. Dietary antioxidants, including polyphenolic compounds, vitamins E and C and carotenoids, are believed to be the effective nutrients in the prevention of these oxidative stress related diseases (Huang, Ou, & Prior, 2005). For this reason, an important effort has been dedicated to identify potential antioxidant-rich cultivars and genotypes for breeding programs (Soengas Fernández, 2011). Plants in the family Brassicaceae are among the oldest cultivated plants known to man. Evidence has been unearthed that indicates that a Brassica vegetable was widely cultivated as early as 10,000 years ago (Snowdon, Luhrs, & Friedt, 2007).

Among plant foods with health benefits, crops from the family *Brassicaceae* have been the focus of numerous epidemiological and clinical studies (Podsędek, 2007). Cruciferous vegetables, those included into the *Brassica* genus, are good sources of a variety of nutrients and health promoting phytochemicals. It has been demonstrated that a high intake of *Brassica* vegetables reduces the risk of age-related chronic illnesses such as cardiovascular health and other degenerative diseases (Kris-Etherton et al., 2002) and reduces the risk of several types of cancer (Björkman et al., 2011). *Brassica oleracea* var. Italica is closely related to cauliflower since both are grown for the clusters of unopened flower buds and tender flower stalks (Stephens, 1994). *Brassica oleracea* var. Italica is popularly used as food and has many traditional claims for herbal medicine (Soengas Fernández, 2011). Based on *Brassica oleracea* var. Italica use in traditional practice and the literature references, the present study was undertaken to evaluate the comprehensive Phytochemicals of *Brassica oleracea* var. Italica and is reported hereunder.

MATERIALS AND METHODS
Collection and identification of plant materials
The whole plants of *Brassica oleracea* var. Italica was collected in the month of June 2012 from the cultivated...
Table 1: Solubility test for hydro ethanolic extract of Brassica oleracea var. Italica in different solvents.

<table>
<thead>
<tr>
<th>Solvent (w/v)</th>
<th>Distilled water</th>
<th>Ethanol</th>
<th>Normal saline</th>
<th>(DMSO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1mg/ml</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
</tr>
<tr>
<td>3mg/ml</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
</tr>
<tr>
<td>6mg/ml</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
</tr>
<tr>
<td>9mg/ml</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
</tr>
<tr>
<td>12mg/ml</td>
<td>Soluble</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
</tr>
<tr>
<td>15mg/ml</td>
<td>Soluble</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
</tr>
<tr>
<td>18mg/ml</td>
<td>Soluble</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
</tr>
<tr>
<td>21mg/ml</td>
<td>insoluble</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
</tr>
</tbody>
</table>

* DMSO: Dimethyl sulfoxide.

Table 2: Phytochemical analysis of hydro ethanolic extract of Brassica oleracea var. Italica.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Chemical compounds</th>
<th>Test name</th>
<th>Observations</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>Pale ppt formed</td>
<td>present</td>
</tr>
<tr>
<td>2</td>
<td>Saponins</td>
<td>Froth test</td>
<td>Stable persistent froth about 1.1cm</td>
<td>present</td>
</tr>
<tr>
<td>3</td>
<td>Reducing sugars</td>
<td>Fehling test</td>
<td>Brick red ppt observed</td>
<td>Present</td>
</tr>
<tr>
<td>4</td>
<td>Phenols</td>
<td>Ferric chloride test</td>
<td>Bluish color formed</td>
<td>present</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>Lead acetate test</td>
<td>Red ppt observed</td>
<td>present</td>
</tr>
<tr>
<td>6</td>
<td>Flavonoids</td>
<td>Alkaline reagents test</td>
<td>Reddish pink colors observed</td>
<td>presents</td>
</tr>
<tr>
<td>7</td>
<td>Resins</td>
<td>Acetone water test</td>
<td>Clear solutions observed</td>
<td>absent</td>
</tr>
<tr>
<td>8</td>
<td>Terpenoids</td>
<td>Salkowaski test</td>
<td>Reddish brown color observed</td>
<td>presents</td>
</tr>
<tr>
<td>9</td>
<td>Anthraquinones</td>
<td>Bontrager’s test</td>
<td>No pinks colors observed</td>
<td>absent</td>
</tr>
</tbody>
</table>

*ppt: precipitate

field in Selangor, Malaysia. The plant was authenticated by Ms. Tan Ai Lee at Forest Research Institute Malaysia and a voucher specimen herbarium with number (SBID: 014/14) was deposited at the Faculty of Pharmacy, Lincoln University College, Malaysia.

Preparation of the plant extract

After shade drying, the plant of Brassica oleracea var. Italica were grinded through mechanical grinder and converted into coarse powder and then ethanolic extract of the plant was made through soxhlet extraction process using 200gm of the dry powder in 95% of ethanol for 48 hrs. After extraction, the extract was concentrated under reduce pressure through rotary evaporator (N-10000, EYELA, Japan), operated at 25°C and then 40°C throughout the process and air dried. The dried powder obtained was 15gm (7.5%) on dry weigh basis which was calculated by using following equation:

Percentage yield = \( \frac{\text{Weight of dried extracts}}{\text{Weight of powder taken}} \times 100 \)

The dry extract was placed in desiccator to avoid moisture and for further pharmacological studied.

Solubility study of crude extract

A series of solvents, including distilled water, normal saline, DMSO, and ethanol was used for checking the solubility of extract (Table 1). The solubility of the extract was determined by increasing the concentrations three folds. The concentration was increased gradually until the extract was not soluble. Gentle heating on water bath and shaking was also applied to allow maximum solubility of extract in solvent. It was noted that hydro ethanolic extract of Brassica oleracea var. Italica was soluble in ethanol (up to 18mg/ml) and further high concentration it became insoluble. In normal saline, DMSO and distilled water the extract was freely soluble even at high concentration up to (21mg/ml).

Qualitative analysis for phytochemical components

A small portion of the hydro ethanolic extracts of Brassica oleracea var. Italica was subjected to the phytochemical test using the standard methods (Trease and Evans and Harbourne) to test for the presence of alkaloids, tannins, reducing sugars, saponins, terpenoids, phenols, flavonoids and Anthraquinones (Harborne, 1998; Trease GE, 1983).

Test for presence of Alkaloids

For the presence of an alkaloid, Mayer’s test was used.0.5gm of extract was taken and diluted in 10ml of 1% of hydrochloric acid. The mixture was then boiled for 2 minutes on water bath and filtered. The filtrate was separated into portions. From the portion, 1ml was taken in a test tube and few drops of Mayer’s reagent were added to the solution. Formation of the pale precipitate was considered the positive test for the presence of alkaloids.

Test for the presence of Saponins

For the presence of saponins in extract froth test was performed. An amount of 0.2gm of extract was taken in a test tube that contains 5ml distilled water, shacked vigorously. Formation of stable persistent for about 05 minutes indicates the presence of saponins.

Test for the presence of reducing sugars

IJPRR, Volume 9, Issue 7: July 2017
For the presence of reducing sugars in extract Fehling test was performed. An amount of 0.2gm of extract was taken and added it to the equal volume of boiling Fehling solutions (A and B) in a test tube. A brick-red precipitates indicates the presence of reducing sugar.

**Test for presence of Tannins**

An amount of 0.1gm of extract was diluted in 5ml distilled water in a test tube, and then added few drops of 1% lead acetate solution. A red precipitate shows the presence of tannins.

**Test for presence of Phenols**

The presence of phenols in extract was accessed by Ferric chloride test. An amount of 0.2gm of extracts was mixed with 1ml of absolute ethanol in a test tube, and then adds few drops of 10% ferric chloride solutions. The bluish colors formations showed the presence of phenols.

**Test for presence of Resins**

Water acetone test was performed for the presence of resins. An amount of 2ml of extract was treated with ethanol. Then small amount of D/W was added and shaken well. Turbidity indicates the presence of resins.

**Test for Terpenoids**

Salkowski test was used to determine the presence of terpenoids in extract. 2ml of chlorof orm was taken in a test tube, and then added 0.5gm of extract, mixed thoroughly. Add 3ml of concentrated Sulphur acid carefully to form a layer. A reddish-brown coloration showed the presence of terpenoids.

**Test for the presence of Flavonoids**

A 0.2 g of the extract was dissolved in 2 ml of methanol and heated. A chip of magnesium metal was added to the mixture followed by the addition of a few drops of concentrated HCl. The occurrence of a red or orange coloration was indicative of the flavonoids.

**Test for the presence of Anthraquinones**

An amount of 0.5gm of extract was taken in 10ml of boiled sulphuric acid, filtered when hot. The filtrate was shacked with 5ml of benzene. The benzene layer was separated and put it into another test tube; 1ml of 10% ammonia solution was added into it, pink color was appeared in the lower layer of ammonia, which showed the presence of Anthraquinones.

**RESULT AND DISCUSSION**

The phytochemicals screening on extract was done for the presence or absence of alkaloids, saponins, reducing sugars, tannins, flavonoids, phenols, terpenoids and Anthraquinones. Mayer’s reagents test confirmed the presence of alkaloids. Pale precipitate was formed that showed that the test for the presence of alkaloids was positive (Table 2).

The presence of saponins was determined by froth test, stable persistent froth about 1.1cm was formed showing the presence of saponins and thus the test for saponins was positive. Lead acetate test formed yellow precipitate, indicating the presence of tannins in extract. No turbidity was formed by lead water test, indicating the absence of resins. The presence of phenols was carried out by ferric chloride test. Bluish color was formed, showing the presence of phenols. The presence of terpenoids was assessed by Salkowski test. A reddish pink color was formed, showing that the test for the presence of terpenoids was positive. Appearance of yellow colors indicating the test for presence of flavonoids was positive. Fehling test was performed for the presence of reducing sugars, the formations of brick red precipitate was appeared showed the presence of reducing sugars. Anthraquinones presence was determined by Bontrager’s test, in ammonia phase no pink or violet color was observed, indicating the absence of Anthraquinones.

**CONCLUSION**

Thus, from the present study the plant leaf extracts of Brassica oleracea var. Italicia showed an abundant production of Phytochemicals as secondary metabolites and they can be used in the pharmaceutical industries for producing a potent drug. The studies result of the above two plants gives a basis of its use in traditional medicine to manage ailments and disorders.

**CONFLICT OF INTEREST STATEMENT**

The authors of this paper have no conflict of interests.

**ACKNOWLEDGEMENT**

The authors would like to acknowledge Lincoln University College Malaysia for laboratory facilities and to facilitate to use animal experimental facilities of the university. The authors also acknowledge the valuable insight of research colleagues, Lincoln University College, Malaysia and OPJS University, Churu, Rajasthan.

**REFERENCES**

