

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

Shah M A^{1*}, Himanshu¹, Sarker M M R^{2,3}, Banu Afreen⁴

¹Department of Microbiology, OPJS University, Churu, Rajasthan. Email¹:

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, Dhanmondi, 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 Anthraquinones. **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, Lühs, & 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.

Extract: Solvent (w/v)	Solubility			
	Distilled water	Ethanol	Normal saline	(DMSO)
1mg/ml	Freely soluble	Freely soluble	Freely soluble	Freely soluble
3mg/ml	Freely soluble	Freely soluble	Freely soluble	Freely soluble
6mg/ml	Freely soluble	Freely soluble	Freely soluble	Freely soluble
9mg/ml	Freely soluble	Freely soluble	Freely soluble	Freely soluble
12mg/ml	Soluble	Freely soluble	Freely soluble	Freely soluble
15mg/ml	Soluble	Freely soluble	Freely soluble	Freely soluble
18mg/ml	Soluble	Freely soluble	Freely soluble	Freely soluble
21mg/ml	insoluble	Freely soluble	Freely soluble	Freely soluble

* DMSO; Dimethyl sulfoxide.

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

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

*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, shaken vigorously. Formation of stable persistent for about 05 minutes indicates the presence of saponins.

Test for the presence of reducing sugars

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 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

Salkowaski test was used to determine the presence of terpenoids in extract. 2ml of chloroform 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 layered 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 Salkowaski 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. 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.

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

1. Anderson, K. J., Teuber, S. S., Gobeille, A., Cremin, P., Waterhouse, A. L., & Steinberg, F. M. (2001). Biochemical and Molecular Action of Nutrients. *J. Nutr*, 131, 2837-2842.
2. Björkman, M., Klingen, I., Birch, A. N., Bones, A. M., Bruce, T. J., Johansen, T. J., . . . Smart, L. E. (2011). Phytochemicals of Brassicaceae in plant protection and human health—Influences of climate, environment and agronomic practice. *Phytochemistry*, 72(7), 538-556.
3. Harborne, J. B. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*: Springer Science & Business Media.
4. Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of agricultural and food chemistry*, 53(6), 1841-1856.
5. Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., . . . Etherton, T. D. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *The American journal of medicine*, 113(9), 71-88.
6. Podsedek, A. (2007). Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. *LWT-Food Science and Technology*, 40(1), 1-11.
7. Snowdon, R., Lühs, W., & Friedt, W. (2007). Oilseed rape *Oilseeds* (pp. 55-114): Springer.
8. Soengas Fernández, M. d. P., Sotelo Pérez, T., Velasco Pazos, . (2011). Antioxidant properties of Brassica

- vegetables. . *Functional plant science and biotechnology*, 5 (2), 43-55.
9. Stephens, J. M. (1994). *Bambara Groundnut--Voandzeia Subterranea L. Thouars*: University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS.
10. Trease GE, E. W. (1983). *Pharmacognosy. . Publ. Brown, 14th Edt, .*