CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com *Research Article*

A CORRELATION STUDY OF ANTIOXIDANT POTENTIAL'S FROM *SYNAPIS ALBA*

Joanne Thangi[1], K. N. Shashitha[1], Ashwini . H. A[1], Shlini. P[2*]

[1,2]Department of Chemistry (PG Biochemistry). Mount Carmel College, Palace Road, Bengaluru - 560 052, Karnataka. India.

Abstract :

Synapis alba commonly known as White Mustard belongs to the family Cruciferaceae. These plants are indigenous to Europe, flowering occurs from June to August. White mustard seen is about 2mm in diameter, globular, testa yellowish, finely pitted, hard, embryo oily. The present work has been carried out to evaluate and extract the potent protein and phenolic antioxidant of aqueous extract of Synapis alba. The potent protein and phenolic content of Synapis alba were investigated spectrophotometrically by using Folin-ciocalteau reagent and the antioxidant activity was determined using ferric reducing power assay. The presence of phenolics was also detected by TLC. Statistical data exhibited a positive correlation of phenolic to the antioxidant activity which was significant and comparable to the standard antioxidant ascorbic acid. The results of the present work suggest that the seeds of Synapis alba possess potent antioxidant property which is a good source of natural antioxidant which can be used for a potent antioxidant drug.

Keywords: *Antioxidant, Phenolic, Synapis alba, Ascorbic acid.*

Corresponding author:

Shlini. P,

Department of Chemistry (PG Biochemistry),
Mount Carmel College. Palace Road,
Bengaluru - 560 052. Karnataka. India.
Email:shlini_p@rediffmail.com

QR code

Please cite this article in press as Shlini .P et al, **A Correlation Study of Antioxidant Potential's from *Synapis alba*** *, Indo Am. J. Pharm. Sci, 2016; 3(3).*

INTRODUCTION:

Synapis alba is commonly known as White Mustard. It is sometimes referred to as *Brassica alba* or *B. hirta*. It is an annual plant, with a thinly hirsute stem, 2 to 5 feet high, which belongs to the family Brassicaceae [1]. The leaves are smoothish, lyrately pinnate, irregularly dentate, rugged, and pale-green; the lower lobes oblong and deeper; the terminal larger. Flowers large, pale-yellow; petals ovate, with straight claws; sepals linear, green, equal at base, and spreading. The pods are spreading, hispid, torose at the place of the seeds, nerved, shorter than the compressed, ensiform beak, about 4-seeded. The seeds are globose, large, and pale.

It is believed that mustard was first cultivated in India around 3000BC. Mustard is a well-known spice that has a long history of medicinal use in stimulating internal and external applications [2].

Synapis alba prefers a light well-drained soil and sunlight. It flowers from June to August and the seeds ripe from July to September. Mustard seeds generally take three to ten days to germinate if placed under the proper conditions, which include a cold atmosphere and relatively moist soil. Mature mustard plants grow into shrubs. Mustard seed is a rich source of oil, protein and minerals. The seeds of *Synapis alba* contains 27.2g of protein, 35g of fat, 34g of carbohydrate, 6g of fiber and 4.5g of ash. Mineral contents include 500mg of calcium, 800mg of phosphorus, 16mg of iron, 5mg of sodium and 732mg of potassium. It also contains 400mg of vitamin A, 0.5mg of thiamine, 0.37mg of riboflavin and 8mg of niacin [3].

The entire seed is employed in medicine as the seeds have the following medicinal uses: antibacterial, antifungal, appetizer, carminative, diaphoretic, digestive, diuretic, emetic, expectorant, rubefacient and stimulant. It is used in small quantities, internally, as a condiment and mild but efficient excitant of the organs of digestion. It helps to regulate irregular heartbeat, relieve respiratory congestion, cholesterol and blood sugar levels because of its magnesium content. It is also used in the treatment of respiratory infections, arthritic joints, chilblains and skin eruptions etc. The seed has an inhibitory action on the growth of fungus [4].

There is a wide variety of phenolic compounds in mustard seeds which includes esterified and free forms of phenolic acids.

The present study is carried out to extract and estimate the proteins, phenolics and antioxidant activity from the seeds of Sinapis alba using aqueous medium. The antioxidant activity of extract was evaluated by reducing power assay and the phenolics and proteins were estimated by FC method.

MATERIALS AND METHODS:

Chemicals:

The chemicals used were Acetic Acid (AR), Acetonitrile (HPLC), Alkaline Copper Reagent, Anhydrous Sodium Carbonate, Ascorbic Acid, Bovine Serum Albumin, Coomaric Acid (AR), Copper Sulphate, Ethanol, Ferric Chloride, Folin Ciocalteau Reagent, Gallic Acid, Gentisic Acid, Potassium Ferricyanide and Sodium Hydroxide.

Plant Source:

Pure yellow mustard seeds obtained from Punjab (India) was used for all extraction experiments.

It was identified and authenticated by experts from Botanical Survey of India, Pune. The dried seeds were stored in airtight containers until use.

Sample Preparation:

1%, 5%, 10% extracts were prepared by homogenizing the seeds in reverse osmosis water. It was homogenized for half an hour followed by filtration[5,6]. The filtrate was subjected to centrifugation at 8000 rpm for 10 minutes at 4˚C. The extract obtained was stored in airtight containers at 4˚C until use.

Estimation of Total Phenolic Content:

The total phenolic content of the *Synapis alba* extract was determined by using Folin-Ciocalteu reagent following a slightly modified method of Ainsworth [7]. Gallic acid was used as a reference standard for plotting calibration curve. The reaction mixture consists of 1mL of the aqueous extract was mixed with 0.5mL of the Folin-Ciocalteu reagent (diluted 1:1 with de-ionized water). The tubes were allowed to stand for 3 minutes and were neutralized with 2mL of sodium carbonate solution (20%, w/v) incubated at room temperature for 60 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 650nm using a colorimeter. The total phenolic contents were determined from the linear equation of a standard curve prepared with Gallic acid. The content of total phenolic compounds expressed as mg/g Gallic acid equivalent (GAE) of the sample.

Estimation of Total Protein Content:

The total protein content of the *Synapis alba* extract was determined by using Folin-Ciocalteu reagent following a slightly modified method of Lowry-*et-al* [8, 9]. Bovine serum albumin (BSA) was used as a reference standard for plotting calibration curve. A volume of 1mL of the aqueous extract was mixed with 5mL of Alkaline Copper reagent, mixed well and allowed to stand for 10 minutes. Followed by addition of 0.6mL of the Folin-Ciocalteu reagent

(diluted 1:1 with de-ionized water) and was incubated for 30 minutes for color development. The absorbance of the resulting blue color was measured at 660nm using a colorimeter. The total protein contents were determined from the linear equation of a standard curve prepared with BSA. The content of total protein was expressed as mg/g of the sample.

Estimation of Total Antioxidant Activity:

The total antioxidant content of the *Synapis alba* extract was determined by using Ferric reducing power assay by the method of Hinneburg *et al*, 2006 with slight modifications. Gallic acid was used as a reference standard for plotting calibration curve. The reaction mixture consists of 1mL of the aqueous extract was mixed with 0.5mL of Potassium Ferricyanide (1.0%). The tubes were incubated at 50°C for 20 minutes, 0.5ml of 10% Trichloro acetic acid was added to the mixture and centrifuged for 10 minutes at 6000rpm. The pellet was discarded. To the supernatant 0.1ml of water and 0.1ml of Ferric chloride (0.1%) was added. The absorbance of the resulting blue color was measured at 700nm using a colorimeter. The total antioxidant contents were determined from the linear equation of a standard curve prepared with Gallic acid. The content of total antioxidant compounds expressed as mg/g Gallic acid equivalent (GAE) of the sample.

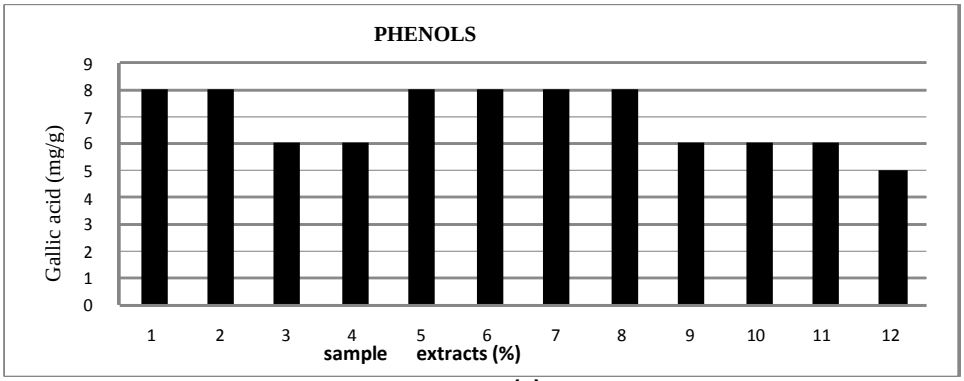
Statistical Analysis:

Samples were quadruplated to estimate the total phenol and protein content along with the total antioxidant activity. The statistical data obtained was used to correlate phenols and protein with its antioxidant activity respectively using Karl Pearson's Co-efficient of Correlation.

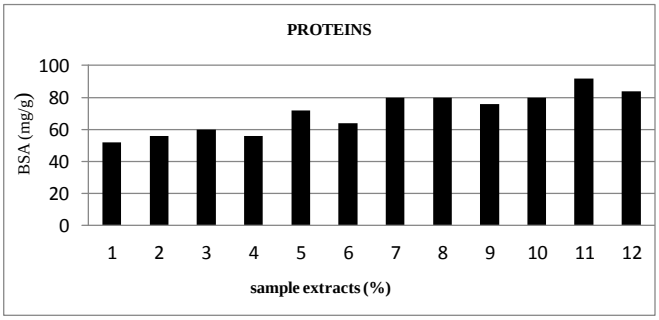
RESULTS AND DICUSSION:

Total Phenolic Content:

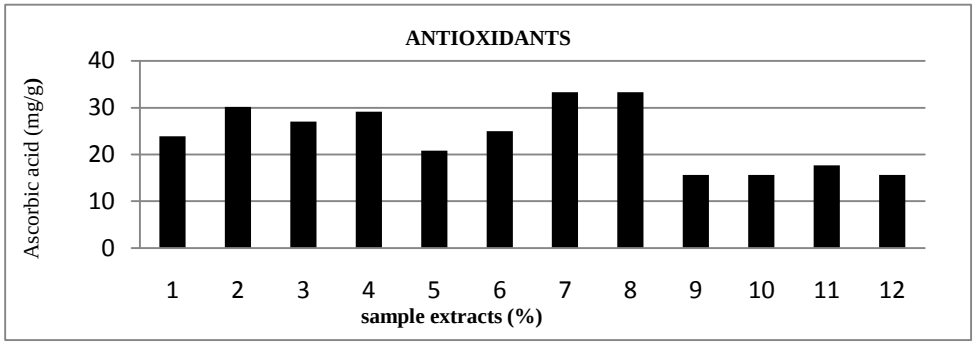
The total phenolic content per gram of yellow mustard seeds for 1% extracts was found to be 4, 6 and 8 mg/g respectively. The 5% extracts of *Synapis alba* were found to contain maximum phenolic compounds when compared to 1% and 10% extracts. In the fig 1 (a) given below, sample extracts- 1,2,3,4 correspond to 1% extract, 5,6,7,8 correspond to 5% extract, and 9,10,11,12 correspond to 10% extract respectively. The phenolic compounds in yellow mustard seeds are usually found as methoxylated derivatives of 19 benzoic and cinnamic acids, which is responsible for the peculiar astringent taste used for the tastemaker. These phenolic compounds may be responsible for the antioxidant property of yellow mustard.



(a)



(b)



(c)

Fig 1: Extraction and Estimation of a) Proteins b) Phenolics and c) Antioxidants from Sinapsis alba (1%, 5%, 10%) using Aqueous Solvent System

The phenolic compounds are usually found as methoxylated derivatives of 19 benzoic and cinnamic acids. The most abundant phenolic compounds present in yellow mustard are p-hydroxybenzoic acid and sinapic acid, present also as sinapine, its choline ester form [10]. Phenolic compounds are known to have a strong antioxidant effect, but are also responsible for a bitter and astringent taste in the mustard seed meal as well as a dark color [11].

Total Protein Content:

The total protein content per gram of yellow mustard seeds for 1% extracts were found to be 52, 56, 60, 56 mg/g respectively. 5% extracts contained 72, 64, 80 and 80 mg/g respectively. The highest protein content was found to be in 10% extract having 76, 80, 90, 84 mg/g respectively. In the fig 1 (b) given , sample extracts-1,2,3,4 correspond to 1% extract, 5,6,7,8 correspond to 5% extract and 9,10,11,12 correspond to 10% extract respectively.

Mustard seeds are known to be rich sources of protein content having about 27.2 g% by AOAC method of analysis (1995). The mustard seeds when consumed were also found to enhance the antioxidant activity of enzymes such as super oxide dismutase, catalase and glutathione peroxidase, hence these proteins may be responsible for the antioxidant property seen in mustard seed extract.

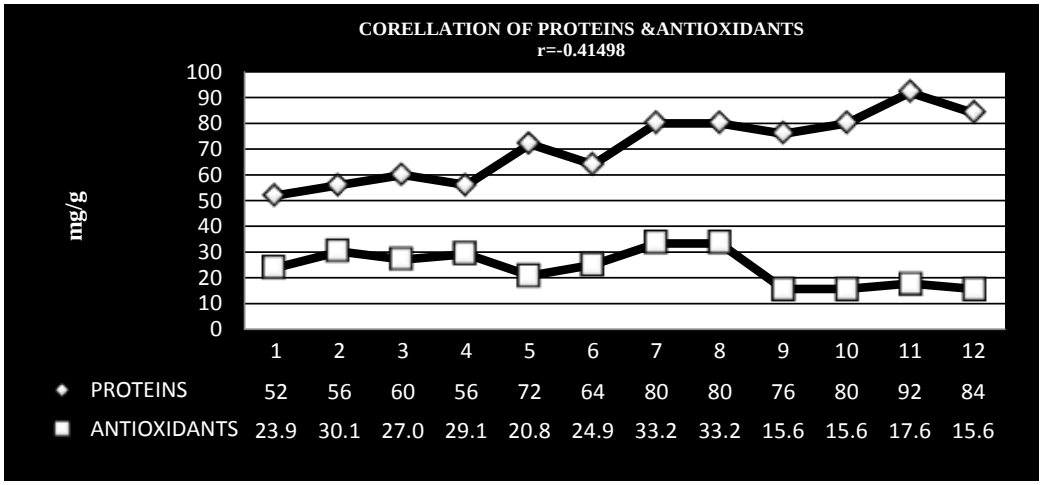
Total Antioxidant Content:

The total antioxidant content per gram of yellow mustard seeds was found to be 23.92, 30.16, 27.04, 29.12 mg/g respectively for various 1% sample extracts. The 5% sample extracts was found to have maximum antioxidant activity having antioxidant content of 20.8, 24.96, 33.28, 33.28 mg/g respectively. The 10% extracts contained about 15.6 and 17.68 mg/g respectively. In the fig 1 (c) sample extracts -1, 2, 3, 4 correspond to 1% extracts, 5,6,7,8 correspond to 5% extract and 9,10,11,12 correspond to 10% extract respectively.

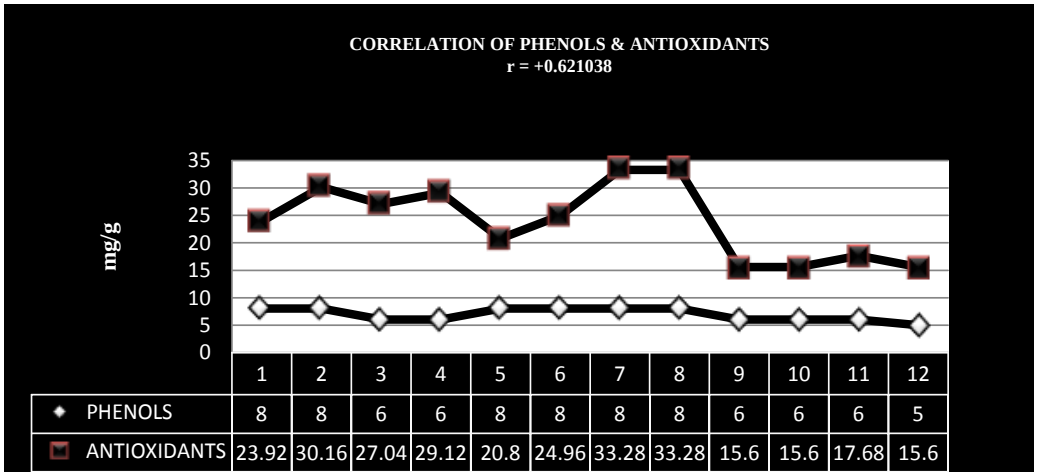
The antioxidant property of *Synapis alba* may correspond to a protein origin such as glutathione, or even a phenolic origin such as sinapic acid, tannic acid and so on. Correlation studies are carried out to find out whether protein or phenol compounds are responsible for the above antioxidant activity.

Correlation of Phenols and Protein With The Antioxidant Activity:

Correlation of phenols and proteins was carried out with the help of Karl Pearson's Coefficient of Correlation (r) to find out the source responsible for the antioxidant activity in aqueous extract of mustard seed. It was found that the phenols had a positive correlation (+0.621038) to the antioxidant activity as shown in fig 2 (b) whereas the proteins were found to have a negative correlation (-0.41498) with the antioxidant activity as shown in fig 2 (a). This infers that the antioxidant activity seen in our yellow mustard sample was found to be a phenolic compound. This finding corresponds to the literature survey carried out before; the phenolic compound responsible for this antioxidant activity may be sinapic acid, tannic acid, gallic acid and so on.



(a)



(b)

Fig 2: Correlation graph of - (a) Proteins with antioxidants (b) Phenols with antioxidants.

SUMMARY:

In recent years there is great demand for antioxidants from natural sources. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables which are consumed on daily basis. The mustard seeds are commercially available and easily cultivated in India and in many traditional practices mustard is used as a tastemaker which is commonly used in pickling, seasoning of food, preparation of oriental sauces and it has become an inevitable primary ingredient in preparation of dishes.

Currently, many studies devote to explore and utilize natural antioxidants to remove excessive free radicals in human body, thus realizing the prevention and treatment of many diseases, which are highly correlated with free radicals and cellular redox imbalance. According to our current study the seeds of *Synapis alba* have proven to have high antioxidant activity, of which most of the antioxidant compounds belongs to the phenolic group such as p-hydroxybenzoic acid, sinapic acid, sinapine, etc.

With the results obtained from various assays we could conclude that *Synapis alba* is a potent natural

antioxidant which can be consumed for the treatment of diseases involving free radicals such as protection against cancer, reducing the buildup of atherosclerotic plaque, delaying some effects of aging and decreasing the chance of cataract formation in the lens of the eye and other damages to the DNA and the cellular structures.

ACKNOWLEDGEMENT:

The authors wish to acknowledge Department of Chemistry (PG Biochemistry) and the management of Mount Carmel College Autonomous, Bengaluru for funding this project and offering their facilities for the analysis.

REFERENCES:

1. Webb, D.A., Parnell, J. and Doogue, D. 1996. "An Irish Flora", pg 15. Dundalgan Press Ltd., Dundalk, ISBN 0-85221-131-7.
2. Wood, Rebecca. 1988. The Whole Foods Encyclopedia. New York, NY: Prentice-Hall Press; PMID: 15220.
3. Cooke, MarcusS; Mark D. Evans; MiralDizdaroglu and Joseph Lunec. "Oxidative DNA damage: mechanisms, mutation, and disease". The Journal of the Federation of American Societies for Experimental Biology. 2014; Vol, 17. pg: 1195-12.
4. Grieve, M. A modern herbal. Reprint 1974. Hafner Press, New York.
5. Balke, D. 2000."Rapid aqueous extraction of mucilage from whole white mustard seed". Food Research International 33 (5) pg: 347–356
6. Ataya Pulido, Veronica Maria., 2010. The production of a potential feedstock for biodiesel using water and isopropyl alcohol to extract yellow mustard oil. Toronto: M. A. Sc. thesis, Chemical Engineering and Applied Chemistry, University of Toronto.
7.Ainsworth, E.A., Gillespie, K.M., 2007, Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent, *Nat Protoc*., 2(4):8757.
8. Troxler, 1981.J. Intoxication mortelle de 19 genisses par la moutarde jaune (*Sinapis alba*) Sweizer Archiv Fur Tierheil Kunde 123(9):495–497.
9. AOAC., 1995, Official methods of analysis of the association of official analytical chemistry 16 thEdn. AOAC International Washington, USA, 1141- 7.
10. Kozlowska, H., Zadernowski, and F. W. Sosulski. 1983."Phenolic acids in oilseed flours." Nahrung 5 pg: 449-453.
11. Shahidi, F., and M. Naczk."Effects of processing on the content of condensed tannins in rapeseed meals." Journal of Food Science 1989; 54, no. 4 pg: 1082-1083.
12. Ensminger AH, Ensminger, ME, Kondale JE, Robson JRK. 1983. Foods & Nutriton Encyclopedia. Pegus Press, Clovis, California.
13.Tuteja N;Singh MB; Misra MK; Bhalla PL and Tuteja R. 2014. "Molecular mechanisms of DNA damage and repair: progress in plants".PubMed.gov.4.2001. pg: 337-97.
13.Barzilai A and Yamamoto K. 2014. "DNA damage responses to oxidative stress". PubMed.gov. pg: 8-9.
14. Gorelov, E.P., Rasulov, I.R., and Odilov, S.K. 1980.Fresh fodders in spring (Russian). Kormoproizvodstvo pg: 34,
15.Yuan , Haifeng ; Minggu Zhu; Wen Guo; Ling Jin; Weihong Chen; Ulf T Brunk and Ming Zhao. 2014. "Mustard seeds (Sinapis Alba Linn) attenuate azoxymethane-induced colon carcinogenesis".Redox Report. vol. 16, pg: 38-44.
16. Balke, David Thomas. 2006. The production of higher value food ingredients from white mustard seed via aqueous extraction. Toronto: Ph. D. thesis, Chemical Engineering and Applied Chemistry, University of Toronto.
17. Fortin, Francois, 1996. The Visual Foods Encyclopedia. Macmillan, New York.