EAS Journal of Pharmacy and Pharmacology
Abbreviated Key Title: EAS J Pharm Pharmacol
ISSN 2663-0990 (Print) & 2663-6719 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-1 | Issue-2 | Mar-Apr-2019 |

Research Article

Preliminary Study of Phytochemical Properties & Antioxidant Activities in Seeds of *Trigonella foenum-graecum*, *Brassica nigra* and *Salvia hispanica* Species

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Abstract: The chosen samples which are *Trigonella foenum-graecum*, *Brassica nigra* and *Salvia hispanica* were extracted using three different solvents of different polarity which are hexane, ethyl acetate and methanol. Phytochemical screening test that has been test include flavonoid test, alkaloid test, triterpernoid/steroid test, saponin test, tannin test, carbohydrate test, reducing sugar test, and phenolic content test. Phytochemical tests on *T. foenum-graecum* showed positive results on alkaloid and flavonoids in hexane and methanol extracts. Moreover, tannins, reducing sugar, carbohydrate and phenolic content indicated in ethyl acetate and methanol extracts. In *B. nigra* sample, methanol extracts showed positive results with presence of alkaloids, flavonoids, tannins, phenolic content, saponins, reducing sugar and carbohydrates. *S. hispanica* showed the presence of alkaloid, flavonoid in hexane extracts. Meanwhile, reducing sugar and carbohydrate revealed in ethyl acetate and methanol extracts. Methanol extract of the three samples were test for DPPH scavenging activities. *S. hispanica* shows the highest scavenging effect on DPPH radical and the samples also exposed to non-enzymatic antioxidant assay for determination of α-tocopherol and carotenoids. For the scavenging effect of DPPH radical revealed that *S. hispanica* has the highest scavenging percentage compared to the other three samples with 39.55%. For non-enzymatic antioxidant assay, determination of α-tocopherol and carotenoids has conducted on the samples revealed that *B. nigra* shows the high antioxidant properties with the high content of α-tocopherol compared to the other samples. Besides that, species with the highest carotenoid content is *B. nigra*.

Keywords: *Trigonella foenum-graecum; Brassica nigra; Salvia hispanica; Phytochemical; Antioxidant.*

1. INTRODUCTION

Natural product can be defined as active chemical compound that produced by a living organism or it commonly in refer to chemical substances found in nature that have distinctive pharmacological effects (David, L. 2005). The usage of bioactive component in some living organism can be either for medicinal purpose or industrial purposes. Home remedies from long time ago were scientifically being research for further information regarding certain bioactive component of particular living organism. The use of natural product especially in healing or treating is an ancient and universal medicine by plays a prominent role in ancient traditional medicine system. The plant derived medicines can be categorised into five major chemicals included alkaloid, flavonoids, terpenes, steroids and others include phenolic compounds. Moreover, there is an increasing interest in studies of antioxidant phytochemicals due to its ability to inhibit the propagation of free-radical reactions and protect the human body from diseases. Free-radicals and other reactive oxygen species (ROS) included superoxide anion, hydroxyl radical, and hydrogen peroxide. This radical may be the causes of kind of diseases such as arthritis, asthma, dementia, mongolism, carcinoma and Parkinson's disease (Lin, C. *et al.*, 2009).

Trigonella foenum-graecum with common name Fenugreek has its own beneficial as herb, spice and vegetable that is commonly found in Mediterranean region, southern Europe, and western Asia. Fenugreek seeds have smell and the taste is something like maple syrup and the leaves are also eaten in India as a vegetable (www.webmd.com, 2017). In Malaysia,

Quick Response Code

Journal homepage:
http://www.easpublisher.com/easjpp/

Article History
Received: 28.03.2019
Accepted: 12.04.2019
Published: 23.04.2019

DOI: 10.36349/easjpp.2019.v01i02.003

locals are widely used the seeds as one of the spices or additional flavour in their cuisines. A bioactive compound which isolated from the fenugreek extract which is 4-Hydroxyisoleucine is found and known for it's responsible for antidiabetic properties. 4-Hydroxyisoleucine is an unusual nonproteinogenic amino acid that significantly improves lipid profile and glucose-induced insulin release in human (Vinod, D., *et al.*, 2015).

Brassica nigra which is from the family of Brassicaceae are native in Europe and widely cultivate to use for producing of mustard oil. The plants are growing wild as a weed in cultivated fields in the Mediterranean region. The seed of this species is chosen as one of the samples because it has the advantage as an edible spice (Anand, P. *et al.*, 2007). Apart from their roles as food additives and supplements, the species may also be utilized as effective and cheap sources of antibacterial agents for the treatment of bacterial infections (Obi, R. K. *et al.*, 2009). B. nigra seeds are used as a spice. They have also been used to treat rheumatism. The seed oil is used for common cold and arthritis. The seed is also used for relieving water retention (edema) by increasing urine production and increasing appetite (Gomezde Saravia, S. G., & Gaylarde, C. C. 1998).

Chia plant is an annual herbaceous plant that belongs to the Lamiaceae family that is 2 mm length herbaceous plant and globally popular due to its high nutritional and functional values. The chemical composition and nutritional value of the Chia seed can vary according to the climatic. The chia seed seems popular and being consume in some places due to its good function related to obesity, cardiovascular disease, diabetes and some types of cancer (Da Silva, B. P. *et al.*, 2017). Rosmarinic acids was found to be the major compound detected and quantified in chia seeds compared to the other species of the same genus. The second major compound found is Protocatechuic acids that act as a strong antioxidant with antitumor effects or as inducer of apoptosis in human leukaemia cells (Martínez-Cruz, O., & Paredes-López, O. 2014).

2. MATERIAL AND METHOD

All the seeds of *Trigonella foenum-graecum* (fenugreek seed), *Brassica nigra* (mustard seed) and Salvina Hispanica (chia seed) species was taken from local dry market and have been dried before the solvent extraction to make sure there is no water contained in the sample to avoid error.

2.1 SAMPLE PREPARATION

The ground sample was extracted with hexane, ethyl acetate, and methanol. The samples extracted in hexane for one day. The extracts were filtered and the remaining residue is being dried before soak with the next solvent which ethyl acetate. The same step is repeated using methanol. Each of the solvent extract of every sample is evaporate by using rotary evaporation.

2.2 PHYTOCHEMICAL TEST

2.2.1 Alkaloid Test (Wagner's test)

Wagner's reagent is added to 1 g of crude extract and the formation of brown or reddish brown precipitate will indicates the presence of alkaloids.

2.2.2 Flavonoid Test (H_2SO_4 Test)

A few drops of H_2SO_4 are added to 1 mL of crude extracts. The formation of orange colour which indicates the presence of flavonoids will be observed.

2.2.3 Saponin Test (Emulsion Test)

5 mL of distilled water is added to the crude extract of each sample. The solution is then heated until boiled. The formation of stable bubble forth showed the presences of saponin.

2.2.3Tannin Test (Ferric chloride test)

30 ml of distilled water was added to 0.5 g of crude extract of each solvent and then boiled on the heating plate. A few drops of Ferric chloride were then added to the filtrate and being filtered. The formation of dark green precipitate indicates the presence tannin.

2.2.4Carbohydrate Test

0.5 g of a crude extract of each sample was dissolved in distilled water and was then shaken and filtered. Then, the filtrate was boiled with a few drops of Fehling' reagent that added to the mixed solution for a few minutes. The formation of orange red precipitates indicates the presence of reducing sugar compound.

2.2.5Triterpernoid / Steroid Test

20 mL of ethanol is added to 0.5 g crude extract of each of three samples and heated in water bath until boiled. The solution was filtered and the filtrate evaporated to concentrate until dry in water bath. The remaining residue was added with 10 mL of diethyl ether and filter. Then, the filtrate was dry in open air and drop with 2-3 drops of acetic anhydride follow by 1-2 drops of concentration sulfuric acid. The colour changes to blue or green shows the present of steroid while formation of red colour shows the presence of triterpenoid.

2.2.6 Phenolic Content Test

5 ml distilled water is 0.5 g of a crude extract of each solvent and 2-3 drops of 10% iron (III) chloride solution was then added to the mixed solution. The formation of green or blue colour indicated the presence of phenolic compounds.

2.2.6 Reducing Sugar Test

0.5 g of a crude extract of each sample was dissolved in distilled water and was then shaken and filtered. Then, the filtrate was boiled with a few drops

of Fehling' reagent that added to the mixed solution for a few minutes. The formation of orange red precipitates indicates the presence of reducing sugar compound.

2.3 ANTIOXIDANT ACTIVITIES

2.3.1 Scavenging Effect on DPPH Radical

The DPPH scavenging effect activity in the samples is determine by adding 0.25 mL 0.2M DPPH radicals in methanol solution to the 1 mL of methanol crude extracts from each sample. The mixture was shaken vigorously and left to stand for 30 minutes at room temperature. The reduction of DPPH was measured by reading the absorbance at 517 nm. 1 mL of α-tocopherol and BHT were used at 20 M in methanol as controls. The scavenging effect on DPPH radical (%) was calculated using the formula:

Scavenging effect (%) = (A-A1)/A ×100%, (1)

Where A was the absorbance of the control, and A1 was the absorbance of the test sample.

2.3.2 Determination of α-Tocopherol

1.5 mL acetone was added to 0.15 g of ground fresh sample at 0-4 °C in a condition of dim light and over ice. The mixture then was extracted with 0.5 mL hexane followed by vortexes for 30 seconds. The mixture was centrifuged at 10,000 rpm for 10 minutes. After the centrifugation process, the top layer of the mixture was removed and the hexane extraction was repeated twice. Meanwhile, the assay mixture was prepared as follows; 0.5 mL of the hexane extract was added into 0.4 mL of 0.1% (w/v) PDT (3-(2-pyridyl)-5, 6-diphenyl-1, 2, 4-triazine-p in ethanol) and 0.4 mL of 0.1% (w/v) ferric chloride (in ethanol). The volume was made up to 3.0 mL with absolute ethanol and the mixture was gently swirled and left for 4 minutes for colour changes. Next step, 0.2 mL of 0.2 M orthophosphoric acid was added into the mixture and allowed to stand for 30 minutes at room temperature. The absorbance of the mixture was measured at 554nm after the process.

2.3.3 Determination of Carotenoids

3.0 mL 0f 80% (v/v) acetone was added to 0.02 g of ground raw dried samples under condition dim light and over ice then was centrifuged at 10,000 rpm for 10 minutes. The absorbance of the supernatant was measured at 663.2, 646.8, and 470 nm. As a blank, 80% acetone was used. Carotenoids content was calculated using formula:

Ca = 12.25(A 663.2) – 2.79(A 646.8), (2)
Cb = 21.50(A 646.8) – 5.10(A 663.2), (3)
Cx+c= (〖1000A〗_470-1.82Ca-85.02Cb)/198, (4)
Where
Ca: chlorophyll a (mg/L)
Cb: chlorophyll b (mg/L)
Cx+c: carotenoids (mg/mL)

3. RESULTS & DISCUSSIONS

3.1 Phytochemical Tests

The table 1 shows the full results for phytochemical test. All the crude extracts of hexane, ethyl acetate and methanol from three samples were subjected to Alkaloids Test, Triterpenoids/Steroids Test, Flavonoids Test, Saponins Test, Tannin Test Reducing Sugars Test, Carbohydrate Test and Phenolic Content Test.

Table 1: Result of Phytochemical Tests

Test	Hexane extract			Ethyl acetate extract			Methanol extract		
	Tfg	Bn	Sh	Tfg	Bn	Sh	Tfg	Bn	Sh
Alkaloid Test (Wagner's test)	+	+	+	+	+	+	+	+	+
Flavonoid Test (H_2SO_4 Test)	+	-	+	-	-	+	+	+	-
Saponin Test (Emulsion Test)	+	-	-	+	-	-	-	+	-
Tannin Test (Ferric chloride test)	-	-	-	+	-	-	+	+	-
Carbohydrate Test	+	-	-	-	-	+	-	+	+
Triterpernoid / Steroid Test	-	-	-	+	-	-	-	-	-
Phenolic Content Test	-	-	-	+	-	-	+	+	-
Reducing Sugar Test	-	-	-	+	-	-	+	+	-

Key: (+) is positive result, (-) is negative result, Tfg is *Trigonella foenum-graecum*, Bn is *Brassica nigra*, Sh is *Salvia hispanica*

3.2 Scavenging Effect on DPPH radical

The results obtained by performing the scavenging effect on DPPH radical, all plant samples showed positive results toward this test. The highest percentage of scavenging effect among the three samples is *Salvia hispanica* (39.55%) followed by *Trigonella foenum-graecum* (34.20%) and Brassica nigra (36.30%). Table 2 is the summaries of the result of Scavenging Effect on DPPH Radical.

Table 2: Results of Scavenging Effect on DPPH Radical

Sample	Percentage of scavenging effect (%)
Brassica nigra	36.30%
Trigonella foenum-graecum	34.20%
Salvia hispanica	39.55%

3.3 Determination of α-tocopherol

α-tocopherol is a most common and biologically active form of Vitamin E, which is an important natural antioxidant (Engin, K. N. 2008). It is a fat soluble vitamin and potent antioxidant is believed to be important in protecting cells from oxidative stress and regulating immune function. Deetermination of the α-tocopherol content in each of the sample, the sample is measured the absorbance using UV photometric at 554 nm. As for the results, *Brassica nigra* shows the higher antioxidant behaviour with the high content of α-tocopherol (1.05 ± 0.06) whereas sample of *Trigonella foenum-graecum* (0.91 ± 0.07) and lastly *Salvia hispanica* (0.59 ± 0.03). Table 3 shows the result of Determination of α-tocopherol.

Table 3: Result of Determination of α-tocopherol

Samples	Mean ± SD
Brassica nigra	1.05 ± 0.06
Trigonella foenum-graecum	0.91 ± 0.07
Salvia hispanica	0.59 ± 0.03

3.4 Determination of Carotenoids

The antioxidant potential of carotenoids is significance to human health. This is due to the fact that losing antioxidant-reactive oxygen species balance results in oxidative stress, which is a critical factor of the pathogenic processes of various chronic disorders or disease (Fiedor, J., & Science, A. C. 2014). The absorbance obtained was calculated by using formula of carotenoid content. Table 4 shows the result of carotenoid content. Based on the result obtained, the sample species with the highest carotenoid content is *Brassica nigra* (15.09 ± 0.05) while *Salvia hispanica* is the second highest which is and *Trigonella foenum-graecum* (2.73 ± 0.05) has the least carotenoid content (0.11 ± 0.02). Figure 3 shows the comparison of Carotenoid Content.

Table 4: Result of Carotenoid Content

Samples	Mean ± SD
Brassica nigra	15.09 ± 0.05
Trigonella foenum-graecum	0.11 ± 0.02
Salvia hispanica	2.73 ± 0.05

4. CONCLUSION

In conclusion, T. foenum-graecum showed positive results on alkaloid and flavonoids in hexane and methanol extracts. Besides that, tannins, reducing sugar, carbohydrate and phenolic content indicated in ethyl acetate and methanol extracts of this sample. Next, for B.nigra sample, methanol extracts of the sample showed positive results of the presence of alkaloids, flavonoids, tannins, phenolic content, saponins, reducing sugar and carbohydrates. Lastly, S. hispanica revealed that the hexane extract contained showed the presence of alkaloid, flavonoid in hexane extracts. Meanwhile, reducing sugar and carbohydrate revealed in ethyl acetate and methanol extracts. All the samples are contained with the most of the secondary metabolite that being test for. Next, as for the scavenging effect of DPPH radical revealed that S. hispanica has the highest scavenging percentage compared to the other three samples with 39.55%. For non-enzymatic antioxidant assay, determination of α-tocopherol and carotenoids has conducted on the samples revealed that B. nigra shows the high antioxidant properties with the high content of α-tocopherol (1.05 ± 0.06) compared to the other samples. Then, the species with the highest carotenoid content is B. nigra (15.09 ± 0.05).

5. ACKNOWLEDGEMENT

Financial support and facilities for this research was provided by School of Fundamental Sciences, Universiti Malaysia Terengganu, Malaysia. The authors also would like to express their acknowledgment to Ministry of Higher Education Malaysia for research grant FRGS 59391.

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