LGU Journal of
LIFE SCIENCES

LGU Society of Life Sciences

Research Article
Vol 6 Issue 2 April - June 2022

LGU J. Life. Sci
ISSN 2519-9404
eISSN 2521-0130

Extraction of poppy seed (*Papaver somniferum* L.) oil and its antioxidant activity by DPPH Assay

*Muhammad Khalid Saeed, Naseem Zahra, Neelam Shahadat, Kiran Shahzadi, Syed Hussain Imam Abdi, Quratulain Syed

1. Food and Biotechnology Research Centre, PCSIR Laboratories Complex, Lahore, Pakistan

Corresponding Author's Email: rose_pcsir@yahoo.com

ABSTRACT: *The huge mainstream of plants, especially agricultural raw materials possesses extractable oils that may have some commercial value. Poppy seeds have high nutritional value and are used as a source of food and cooking oil. Its oil is rich in polyunsaturated fatty acids. It is well known that polyunsaturated fatty acids not only provide basic nutrients for the body, but their absorption into the body are very important for the prevention of cardiovascular disease and many inflammatory diseases. In this study, poppy seed oil was extracted with a yield of 32.50 ± 1.80% and free radical scavenging activity was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. Antioxidant activity was found to be in the range of 22.28±1.40-58.32±3.40% at concentrations of 20-100 µl. It was concluded in this study that poppy seeds were found as an effective source of natural antioxidants and can be used to substitute synthetic antioxidants.*

Keyword: Poppy seed, Oil content, Antioxidant activity, DPPH assay

INTRODUCTION

Poppy *(Papaver somniferum* L.) is an ancient edible crop that has been used for medical and dietary reasons for ages (Fig. 1). It is grown all over the world and considered as a raw material of prime importance and use for the production of pharmaceutically important drugs and seeds (Lančaričová et al., 2016). Narcotic alkaloids, lipid and oils are absent in poppy seeds.

Because of improper harvesting procedures or the usage of poppy seeds by technological poppy cultivars as a derivate of the cultivation in the

pharmaceutical industries, opium alkaloids may contaminate the final product (López et al., 2009). Several important minerals were present in seeds such as Na, K, Mg, Ca and also the rich amount of omega-6 fatty acids and fibers were reported in it (Levent et al., 2020; Senila et al., 2020).

Fig. 1. Poppy seed plant

(https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:673724-1)

Biomolecule such as vitamin E was present in sufficient quantities as alpha-tocopherol with range of 22.0–45.8 ppm and gamma-tocopherol with range of 195.4–280.9 ppm was reported. Also the Important phytosterol (Beta-sitosterol) was also reported in seeds. Beta-tocopherol (309.5–567.3 ppm) also found in significant concentrations in poppy seed oil (Erinç et al., 2009). Oil content varies from 28 to 52 percent based on seed colour, variety, environmental circumstances, and growing technique (Ghafoor et al., 2019, Luhmer et al., 2021). Fatty acids play a variety of roles in the body. They are precursors of numerous molecules engaged in the processes of energy storage and transport of vitamins as it is the key components of bio-membranes (Petrovi et al., 2010, Labdelli et al., 2019). The major fatty acids in poppy seed oil are polyunsaturated one, particularly linoleic fatty acids, which account for 56.4-74.8 percent of all fatty acids (Bozan and Temelli, 2008). Varied percentage (13.2–17.8%) of several monounsaturated fatty acids were present in seed oil such as 9–2.3% of stearic acid (Valizadeh et al., 2014), 17–0.40% of palmitic acid (Zbek and Ergönül, 2020; Dbrowski et al., 2020) and oleic acid (Rahimi et al., 2011). Synthetic antioxidants impart serious

side effects due to which researchers were focussing on getting natural antioxidants. The majority of natural antioxidants derived from plants, aid in adsorption, neutralisation, and quenching of singlet/triplet oxygen, as well as degrading peroxides (Uttara et al., 2009; Fahad et al., 2021). Several complications can be caused by those reactive oxygen species such as neurological disorders, cardiovascular diseases, diabetes, asthma, cancer and inflammatory disorders, if those reactive species were not captured by oxidant scavenging species i.e. antioxidants (Chan et al., 2011; Lamine et al., 2019; Masooda et al., 2023). Bukhari et al. (2009) and Al-Nemari et al. (2020) have found that a various oil seeds exhibited numerous beneficial benefits, such as demonstrating antioxidant activity. GC-MS analysis of ethanolic extract of *Papaver somniferum L* confirmed the presence of 39 volatile compounds (Fig. 2).

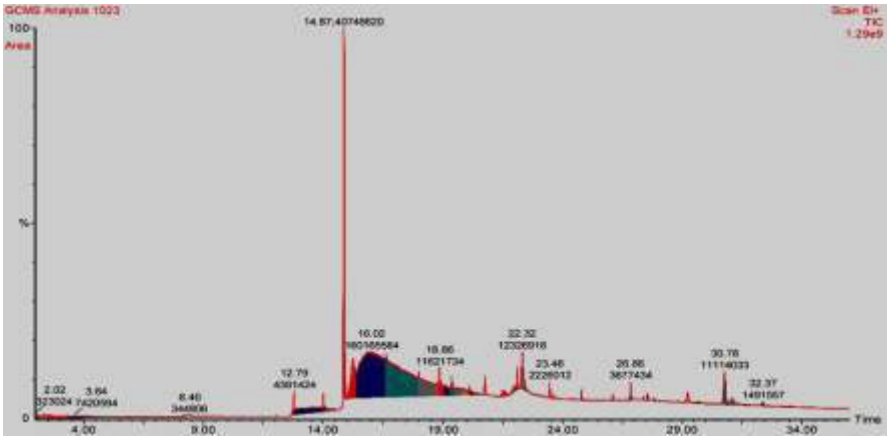

Fig. 2. GC-MS Chromatogram of poppy seed (Muthukumaran et al., 2019)

Different percentage of various phytochemical were reported as follow 30.72% of 9-octadecenoic, 24.02% of 9-tetradecen-1-ol, acetate acid (E), 7.82% of methyl 9,12-octadecadienoate Esters, (E,E), 7.43% of cis-9,10-Epoxyoctadecan-1-ol and 4.36% of Undec-10-ynoic acid. Reported chemical groups are as follow, amino acids (1.42%), monoterpenes (1.33%), fatty acids (51.03%), aromatics (0.47%), nitrogenous compounds (0.14%), acetates (24.31%), alcohols (0.73%), aldehydes (0.33%), alkanes (1.22%), alkenes (1.07%), ketones (0.75%), naphthalenes (0.71%), epoxides (2.23%) and esters (0.94%). Poppy seed oil's quality and application in the culinary business have become increasingly popular in recent years. In this regards

the purpose of current study is to estimate the oil content by using the DPPH technique and to investigate antioxidant effects.

MATERIALS AND METHODS

Chemicals

DPPH (2, 2-Diphenyl-1-picrylhydrazyl) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Analytical graded Methanol and $CHCl_3$ was purchased from local market.

Materials

The poppy seed was purchased from local supermarket Lahore, Pakistan and was dried in an electric oven at 40-50 °C. Grinded it with an agate mortar and pestle to obtain a homogeneous powder.

Oil extraction

The Bligh and Dyer technique 1959 was used to extract the oil using a $CHCl_3$-MeOH combination with the goal of recovering antioxidants from the MeOH-H_2O mixture ensured to minimise the loss by making the procedure more practicable to obtain heat sensitive antioxidants. Powdered material of about 500 g was mixed with 2 liter of $CHCl_3$/MeOH (1:2, v/v) and thoroughly homogenised. After that, 500 ml of $CHCl_3$ was added, homogenised, followed by adding 500 ml of double-distilled water, homogenised again, and lastly subjected to centrifugation for 5 mins at 1000 rpm to generate two phases as $CHCl_3$ on bottom and MeOH-water on top layer. Bottom phase of about 95% was recovered by using separating funnel while the remaining 5% is collected after adding 50-100 mL of $CHCl_3$, shaken vigorously and gathered at the bottom. $CHCl_3$ was evaporated at 40 °C on a rotary evaporator to collect the oil fraction from the chloroform phase. Equation I was used to compute the oil yield in percent.

$$\% \text{ Oil yield} = Wt_{(oil)} / Wt_{(seed)} \times 100 \quad \text{--------} \ (I)$$

Antioxidant study by DPPH assay

Brand-Willium, (1995) method was employed with some modifications to evaluate the free radical scavenging activity by using DPPH reagent (Saeed et al., 2021). About 2 ml of 0.004% of methanolic solution of DPPH was prepared and mixed it with 20-100 µl of poppy seed oil. Shake the mixture thoroughly and incubate for 30 mins and

the absorbance was taken at 517 nm by using UV-Visible spectrophotometer (1700, Shimazdu, Japan). Percentage inhibition of free radical was measured by using Equation II

$$\text{Antioxidant activity \%} = 1 - [A_{sample}/A_{control}] \times 100 \text{ ---------- (II)}$$

Statistical Analysis

Three triplicates of tested sample were utilized to conduct statistical analysis and expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Oil Recovery

In this extraction method, 162.5 g of oil was obtained from 500 g of white poppy seeds and the percent recovery was calculated by using equation-I. The results of oil yield showed that the oil content of poppy seeds was 32.50% in this variety by the above extraction method (Fig. 3). The oil yield generally depends on the quality of the oilseed. However, in order to maximize oil production, certain factors, can control during pre-treatment such as moisture content, particle size and temperature (Yusuf, 2018).

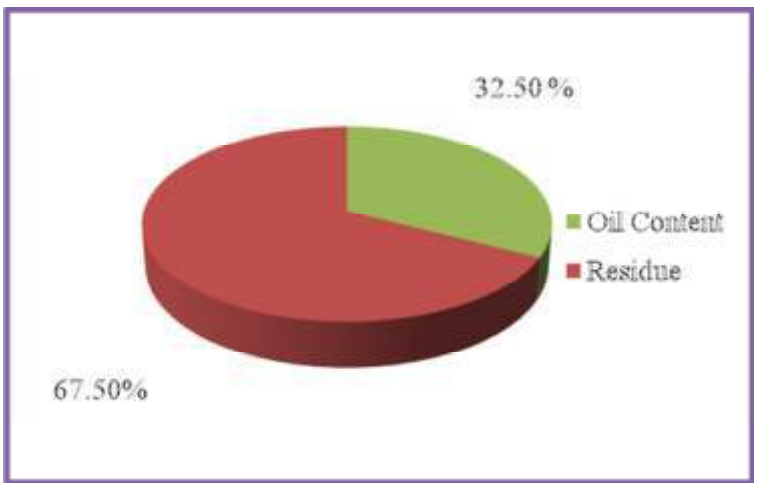

Fig. 3. Percentage yield of oil content of Poppy seed

Solvent extraction is a conventional extraction method commonly used for oilseeds. The choice of solvent is mainly depend on the solutes leaching potential (Dutta et al., 2015). According to Olaniyan (2010), pre-treatment of oilseeds prior to oil extraction often affects quality and oil

yield. Satranský et al. (2021) conducted a study describing the oil content (40.73-44.76%) in white seed genotypes, but in current study, obtained yield was comparatively low suggested to attribute to variations in cultivar, region and extraction method. However, our results are consistent with Özcan and Atalay (2006), who showed that the oil content in poppy seeds ranges from 32.4% to 45.5%, depending on the variety.

Antioxidant activity

Due to the carcinogenic effects of synthetic antioxidants, recent research was focussing on production of natural antioxidants such as found medicinal plants (Huang et al., 2005; Ghosh et al., 2008). Medicinal plants are considered effective source of antioxidants and can be used as an alternative medicine to assuage diseases associated with oxidative stress (Garcia et al., 2007; Afolayan et al., 2011). DPPH antioxidant assay was used to assess the reducing potential of antioxidants in sample (Prior et al., 2005). DPPH•, a free radical, reduced to DPPH in the presence of antioxidants. Analytical conditions cause a decolourization reaction in which the purple DPPH• changes color to yellow upon reduction to DPPH. Spectrophotometer reads the absorbance of the sample at 517 nm to determine the degree of decolourization (Pisoschi et al., 2009). In our study, poppy seed oil quenched DPPH free radicals (Fig. 4) with % inhibition range from 22.28±1.40-58.32±3.40% at concentrations of 20-100 µl. It has been observed that DPPH free radical scavenging activity increases with increasing concentration (Saeed et al., 2022b).

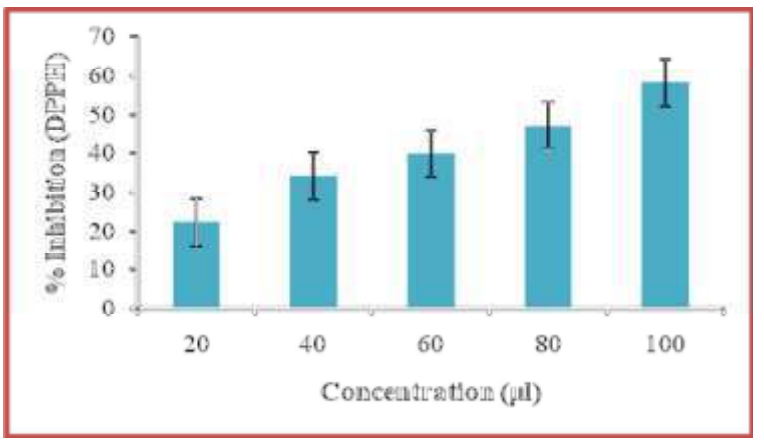

Fig. 4. % Inhibition (DPPH•) of poppy seed oil

Results were in harmony with Chmelová et al. (2018) and Shagufta et al. (2013), who characterized the free radical scavenging activity of poppy seeds in range of 33-56.4% at concentrations 50-250 µg /ml. Chan et al. (2010) reported the antioxidant activity of poppy seeds at concentrations 50-100 µg/100 µl) and the reported activity was high than our study. This may be due to differences in extracting solvents and extraction methods. Higher antioxidant activity attributed to the presence of number of hydroxyl group as it showed correlation such as higher the hydroxyl content, higher will be the antioxidant activity. (Chaieb et al., 2007; Ceriello, 2008; Ahmed Souadia, 2022)

CONCLUSION

According to the findings, poppy seed oil has strong antioxidant properties. Seed oil is an effective source of antioxidants and utilised in very tiny amounts in food. This study also concluded that natural antioxidants suggested effective as compare to synthetic antioxidants. Such natural entities can be used as food preservative and to treat various ailments.

REFERENCES

1. Afolayan AJ, Sunmonu TO, Artemisia AJ (2011). Oxidative stress in the pancreas of streptozotocin-induced diabetic wistar rats. Biosci. Biotech. & Bioche. 75(1): 2083–2086.
2. Ahmed Souadia (2022). Chemical Composition and Antioxidant Activity of *Thymus ciliatus* (Desf.) Benth. Essential Oils of Algeria. Nat. Pro. Commu. 17(2): 1–7.
3. Al-Nemari R, Al-Senaidy A, Semlali A, Ismael M, Badjah-Hadj-Ahmed AH, Abir Ben. Bacha (2020). GC-MS profiling and assessment of antioxidant, antibacterial, and anticancer properties of extracts of Annona squamosa L. leaves. BMC Compl. Med. Ther. 20(296): 1-14.
4. Bligh EG and Dyer WJ (1959). A rapid method for total lipid extraction and purification. Cand. J. Biochem. Physiol. 37(1): 911–917.
5. Bozan B and Temelli F (2008), Chemical composition and oxidative stability of flax, safflower and poppy seed and seed oils. Bio. Tech. 99(1): 6354–6359.
6. Brand-Williams W, Cuvelier ME, Berset C (1995). Use of free radical method to evaluate

antioxidant activity. Food. Sci. Tech. 28(1): 25-30.

7. Bukhari SB, Bhanger MI, Memon S (2008). Antioxidative activity of extracts from fenugreek seeds (*Trigonella Foenum-Graecum*). Pak. J. Anal. Environ. Chem. 9: 78–83.
8. Ceriello A (2008). Possible role of oxidative stress in the pathogenesis of hypertension. Diabe. Care. 31(1): S181–S184.
9. Chaieb K, Zmantar T, Ksouri R, Hajlaoui H, Mahdouani K, Abdelly C, Bakhrouf A (2007). Antioxidant properties of the essential oil of *Eugenia caryophyllata* and its antifungal activity against a large number of clinical Candida species. Myco. 50: 403-406.
10. Chan KW, Iqbal S, Khong NMH, Babji AS (2011). Preparation of deodorized antioxidant rich extracts from 15 selected spices through optimized aqueous extraction. J. Med. Plants Res. 5(1): 6067–6075.
11. Chmelová D, Ondrejovič M, Havrlentová M ,Kraic J (2018). Evaluation of polar polyphenols with antioxidant activities in *Papaver somniferum* L. J. Food Nutri. Res. 57(1): 98–107.
12. Dąbrowski G, Czaplicki S, Konopka I (2020). Composition and quality of poppy (*Papaver somniferum* L.) seed oil depending on the extraction method. LWT – Food. Sci. Tech. 134: e110167.
13. Dutta R, Sarkar U, Mukherjee A (2015). Soxhlet extraction of *Crotalaria juncea* oil using cylindrical and annular packed beds. Int. J. Che. Eng. Appli. 6(2): 130-133.
14. Erinç H, Tekin A, Özcan MM (2009). Determination of fatty acid, tocopherol and phytosterol contents of the oils of various poppy (*Papaver somniferum* L.) seeds. Gras Ace. 60: 375–381.
15. Fahad Y. Al-Juhaimi K, Ghafoor, Mehmet M. Ö, Nurhan Uslu, Elfadıl E Babiker, Isam A. M. Ahmed, Omer N (2021). Alsawmahi. Phenolic Compounds, Antioxidant Activity and Fatty Acid Composition of Roasted Alyanak Apricot Kernel. J. Oleo Sci. 70 (5): 607-613.
16. Garcia MV, Crespo I, Collado PS, Esterller A, Sanchez-Campos S, Tunon MJ, Gonzalez-

Gallego J (2007), The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and downregulation of the nuclear factor kappa B pathway in Chang liver cells. Eur. J. Pharma. 557: 221–229.

17. Ghafoor K, Özcan MM, Juhaimi FY, Babiker EE, Fadimu GJ (2019). Changes in quality, bioactive compounds, fatty acids, tocopherols, and phenolic composition in oven and microwave-roastedpoppy seeds and oil. LWT – Food Sci.Tech. 99: 490–496.
18. Ghosh T, Maity TK, Sengupta P, Dash DK, Bose A (2008). Antidiabetic and In Vivo Antioxidant Activity of Ethanolic Extract of Bacopamonnieri Linn. Aerial Parts: A Possible Mechanism of Action. Iranian J. Pharma. Res. 7(1): 61-68.
19. Hlinková A, Havrlentová M, Šupová J, Bednárová A (2012). Poppy seed (*Papaver somniferum* L.): effect of genotype and year of cultivation on variability in its lipid composition. J. Micro., Biotech. Food. Sci. 1: 908–922.
20. Huang D, Ou B, Prior RL (2005). The chemistry behind antioxidant capacity assays. J. Agri. Food. Chem. 53 (6): 1841-1856.
21. Muthukumaran P, Kumaravel S, Thomas N (2019). Phytochemical, GC-MS and FT-IR Analysis of *Papaver somniferum* L. J. Pharma. Bio. Sci. **7**(1): 1-8.
22. Labdelli A, Zemour K, Simon V, Cerny M, Adda A, Merah O (2019). *Pistacia atlantica* Desf., a source of healthy vegetable oil. Appli. Sci. 9: 2552.
23. Lamine JB, Boujbiha MA, Dahane S, Cherifa AB, Khlifi A, Chahdoura H, Yakoubi MT, Ferchichi S El-Ayeb N, Achour L (2019). A-amylase and α-glucosidase inhibitor effects and pancreatic response to diabetes mellitus on wistar rats of ephedra alata areal part decoction with immunohistochemical analyses. Env. Sci. Pollu. Res. Int., 26(10): 9739-9754.
24. Lančaričová, A, Havrlentová M, Muchová, D, Bednárová A (2016). Oil content and fatty

acids composition of poppy seeds cultivated in two localities of Slovakia. Agri. Pol. 62(1): 19−27.

25. Levent H, Sayaslan A, Yeşil S (2020). Physicochemical and sensory quality of gluten-free cakes supplemented with grape seed, pomegranate seed, poppy seed, flaxseed and turmeric. J. Food. Prod. Preser. 45(1): e15148.
26. López P, Pereboom-de Fauw DPKH., Mulder PPJ, Spanjer M, de Stoppelaar J, Mol HGJ, de Nijs M (2018), Straightforward analytical method to determine opium alkaloids in poppy seeds and bakery products. Food Chem. 242: 443–450.
27. Luhmer K, Schulze-Kaysers N, Feuereisen M, Wirth L, Maretzky F, Wüst M, Blum H, Dörr E, Pude R (2021). Fatty acid composition, tocopherols, volatile compounds, and sensory evaluation of low morphine yielding varieties of poppy (*Papaver somniferum* L.) seeds and oils. J. Agri. Food. Chem. 69: 3439–3451.
28. Masooda S, Atta ur Rehman, Ihsand MA, Shahzade K, Sabirf M, Alamf S, Ahmede W. Shahg ZH, Alghabarih F, Mehmoodi A, Chungj G (2023). Antioxidant potential and α-glucosidase inhibitory activity of onion (*Allium cepa* L.) peel and bulb extracts. Braz. J. Bio. 83: 1-9
29. Olaniyan, AM (2010). Effect of extraction conditions on the yield and quality of oil from castor bean. J. Cer. oil seed. 1:24-33.
30. Özbek ZA and Ergönül PG (2020). Determination of physicochemical properties, fatty acid, tocopherol, sterol, and phenolic profiles of expeller-pressed poppy seed oils from Turkey. J. Amer. Oil Chem. Soci. 97: 591–602.
31. Özcan MM and Atalay Ç (2006). Determination of seed and oil properties of some poppy (*Papaver somniferum* L.) varieties. Gras. Aceit. 57: 169–174.
32. Petrović M, Kezić N, Bolanča V. (2010). Optimization of the GC method for routine analysis of the fatty acid profile in several food samples. Food. Chem. 122: 285–291.
33. Pisoschi, AM, Cheregi MC, Danet AF (2009). Total antioxidant capacity of some

commercial fruit juices: electrochemical and spectrophotometrical approaches. Mol. 14 (1): 480-493.

34. Prior RL, Wu X, Schaich K (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agri. Food Chem. 53(10): 4290-4302.
35. Rahimi A, Kıralan M, Arslan N, Bayrak A, Doğramacı (2011), Variation in fatty acid composition of registered poppy (*Papaver somniferum* L.) seed in Turkey. Ak. Gi. 9: 22–25.
36. Saeed MK, Nisa A, Ahmad I, Hina S, Zahra N, Kalim I, Masood S Syed Q (2021a). Physico-chemical Analysis, Total Polyphenolic Content and Antioxidant Capacity of Yellow Dye Extracted from *Curcuma longa*. Pak. J. Sci. Ind. Res. Ser. B: Biol. Sci. 64(1): 25-29.
37. Saeed MK, Zahra N, Abidi SH, Syed Q, Firdous S, Riaz A (2022b). *In Vitro* Assessment of the Free Radical Scavenging Activity. Proximate and GC-MS analyses of Essential and Fixed oil of *Nigella sativa* from Pakistan. J. Biotech. Biores. 3(3): 000565.1-5.
38. Satranský M, Fraňková A, Kuchtová P, Pazderů K, Capouchová I (2021). Oil content and fatty acid profile of selected poppy (*Papaver somniferum* L.) landraces and modern cultivars. Plant Soi. Environ. 67(1): 579–587.
39. Senila L, Neag E, Cadar O, Kovacs MH, Becze A, Senila M (2020). Chemical, nutritional and antioxidant characteristics of different food seeds. Appl. Sci. 10(1): 1589.
40. Shagufta I, Nasir K, Siddiqui MA, Siddiqi R, Shahina N (2013). Antioxidant potential of the Extracts, fractions and oils derived from oilseeds. Antiox. 2(1): 246-256.
41. Uttara B, Singh AV, Zamboni P, Mahajan RT (2009). Oxidative stress and neurodegenerative Diseases: A review of upstream and downstream antioxidant therapeutic options. Curr. Neuro. 7(1): 65–74.
42. Valizadeh N, Rahimi A, Arslan N (2014). Variation in fatty acid composition of three turkish slit flower opium poppy (*Papaver*

somniferum L.) lines. Int. J. Biosci. 4(1): 268–274.

43. Yusuf AK (2018). A Review of Methods Used for Seed Oil Extraction. Int. J, Sci. Res. **7**(12): 233-238.