International Journal of Secondary Metabolite
2024, Vol. 11, No. 3, 584–591
https://doi.org/10.21448/ijsm.1413509
journal homepage: https://dergipark.org.tr/en/pub/ijsm
Research Article

Examination of the chemical composition and anti-diabetic activities of the oils of *Abelmoschus esculentus*, *Peganum harmala*, and *Aquilaria agallocha* cultivated in Muğla

Yunus Çetintaş[1*], Ayşe Çetintaş[2], Yusuf Sıcak[3], Mehmet Öztürk[4]

[1]Mugla Sitki Kocman University, Food Analysis Application and Research Center, Research Laboratories Center, Muğla, Türkiye
[2]Mugla Sitki Kocman University, Mugla Vocational School, Department of Chemistry and Chemical Processing Technologies, Cosmetic Technology Program, Muğla, Türkiye
[3]Mugla Sitki Kocman University, Koycegiz Vocational School, Department of Herbal and Animal Production, Muğla, Türkiye
[4]Mugla Sitki Kocman University, Faculty of Science, Department of Chemistry, Muğla, Türkiye

ARTICLE HISTORY
Received: Jan. 04, 2024
Accepted: June 22, 2024

KEYWORDS
Anti-diabetic activities,
Fatty acid,
Belmoschus esculentus,
Peganum harmala,
Aquilaria agallocha.

Abstract: In this study, the oils of *Abelmoschus esculentus*, *Peganum harmala*, and *Aquilaria agallocha* grown in different regions of Muğla, Türkiye were obtained using the cold pressing and maceration techniques. The oils were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) to determine their fatty acid compositions. Thirty-seven fatty acids were detected. Palmitic acid ($C_{16:0}$), linoleic acid ($C_{18:2}$), and oleic acid ($C_{18:1}$ *cis*-9) were the major components in all oils. Additionally, the anti-diabetic activity of the oils was screened against α-amylase and α-glycosidase, which are the related enzymes to diabetes mellitus. Promising results regarding anti-diabetic activity for *Aquilaria agallocha* oils were obtained.

1. INTRODUCTION

The prevalence of type 2 diabetes is rapidly increasing worldwide. There is a consensus among researchers and relevant organizations that lifestyle and dietary factors play a role in the development of the disease. Numerous research data suggest that an increase in saturated and trans fats in the diet increases the risk of coronary heart disease and adversely affects glucose and insulin metabolism, thereby increasing the risk of type 2 diabetes (Baysal, 2011).

Fats are composed of saturated and either monounsaturated or polyunsaturated fatty acids. (Lopez *et al*., 2011). Omega-3 and Omega-6 fatty acids are polyunsaturated fatty acids, both metabolically generating numerous long-chain fatty acids that support various physiological and developmental processes in the body. As they are not synthesized again in humans, they

*CONTACT: Yunus ÇETİNTAŞ ✉ yunuscetintas@mu.edu.tr Mugla Sitki Kocman University, Food Analysis Application and Research Center, Research Laboratories Center, Muğla, Türkiye

e-ISSN: 2148-6905

must be obtained through diet. Primary dietary sources include plant seeds, nuts, fish oils, and other seafood. Several studies have demonstrated that omega fatty acids are potent molecules that can reduce the risk of cardiovascular diseases and possess anti-cancer, hypolipidemic, anti-inflammatory, and anti-diabetic activities. Omega-6 fatty acids have several health benefits, and when balanced with omega-3 as a dietary supplement, they play a significant role in preventing degenerative diseases and other inflammatory disorders (Gazem *et al*., 2014).

Okra (*Abelmoschus esculentus* L. Moench) is a crucial vegetable plant. Okra is a popular plant recognized to have various health benefits, including anti-diabetic properties (Dubey & Mishra, 2017). Regarding fatty acids, okra seed oil has been reported to be rich in palmitic, oleic, and linoleic acids (Jarret *et al*., 2011). Syrian rue (*Peganum harmala*) has been traditionally and commonly used for medicinal purposes since ancient times. *P. harmala* is reported to have hypoglycemic and cytoprotective effects (Komeili *et al*., 2016). Udi hindi (*Aquilaria agallocha*), an important medicinal plant, is one of the most grown species in the Thymelaeaceae family. It is a rare plant on Earth due to its medicinal properties. The plant has various pharmacological activities such as antinociceptive, antimicrobial, laxative, antioxidant, sedative, anti-hyperglycemic, thrombolytic, anti-diabetic, ulcer protective, anti-cancer, anti-diarrheal, and hepatoprotective activities (Alam *et al*., 2015). Every part of the plant possesses beneficial properties that can serve humanity. Researchers have investigated the anti-diabetic effect of methanol, water, and hexane leaf extracts of *A. agallocha* on streptozocin-induced diabetic rats. The findings suggest that *A. agallocha* leaves have promising potential as an anti-diabetic agent (Pranakhon *et al*., 2011).

The medicinal use of vegetable oils in areas such as health and nutrition is under consideration. Throughout history, it is known that many vegetable oils have been utilized for medical purposes in both written and traditional practices. Diabetes, a recognized chronic disease known to contribute to the onset of various illnesses, lacks a significant therapeutic agent for treating the condition. This situation not only diminishes the quality of life for individuals with diabetes but also exposes them to the concern of developing a new ailment stemming from diabetes. In this context, this study aims to investigate the anti-diabetic activity of the seed oils of *A. esculentus* and *P. harmala* with *A. agallocha* maceration oil, despite the known medicinal properties of these plant species, which are cultivated in the Muğla province and its regions.

2. MATERIAL and METHODS

2.1. Chemicals

All standards used in the study were obtained from Sigma-Aldrich. Other reagents were of analytical-grade purity and were purchased from Merck. Water was purified using a Millipore Milli-Q system involving reverse osmosis, ion exchange, and filtration steps.

2.2. Preparation of Oils

As a part of the research, *A. esculentus* and *P. harmala* seeds, *A. agallocha* cortex were collected from the Muğla-Marmaris and Köyceğiz regions in the year 2023 during their maturation period. The seeds were dried in a plant dryer. Fixed oils of *A. esculentus* (Aeo) and *P. harmala* (Pho) seeds were obtained by passing each seed through an NF 80 cold press machine (Karaerler, Türkiye). The obtained fixed oils were separated from the pulp by filtering. The sterilized oils obtained were stored in the dark at +4°C before use. *A. agallocha* oil (Aao) was obtained by the maceration technique in olive oil. Seeds visuals of *Abelmoschus esculentus* L. Moench (a), *Peganum harmala* (b), and *Aquilaria agallocha* (c) are given Figure 1.

Figure 1. Seeds of *Abelmoschus esculentus* (a), *Peganum harmala* (b), and *Aquilaria agallocha* (c)

2.3. Determination of Fatty Acid Content by GC/MS

An Agilent 7890A GC-5975C MSD was used to determine the fatty acid content of each oil (AOCS 2007). Fatty acid analyses were conducted in accordance with the literature, with modifications to the method described by Kivrak (2020). Sample specimens, each containing 100 mg of oil, were mixed with 10 mL of *n*-hexane and 100 µL of 2 N methanolic potassium hydroxide (Kivrak *et al*., 2020).

The samples were centrifuged at 4000 rpm for 10 min, and the upper layer was transferred to a new tube. Afterward, it was filtered using a single-use 0.20 µm (Macherey-Nagel Chromafil Extra PTFE-20/25 LC) filter disc. Fatty acid methyl esters were analyzed by gas chromatography-mass spectrometry (GC/MS) using an Agilent 7890 GC 5975C inert MSD (Agilent Technologies, Wilmington, DE, USA).

GC/MS analyses were performed using a multi-mode inlet (MMI) (280°C), equipped with a DB-1 capillary column (30 m x 0.25 mm; 0.25 µm), and coupled with an Agilent 5975C using an Agilent 7890 Gas Chromatography. The Mass Spectrometer (MSD) operated in electron impact (EI) mode at 70 eV. The transfer line temperature was set at 280°C, and the carrier gas was helium (2.1 mL/min), while the oven temperature was held at 110°C for 1 min. It was then raised at a rate of 3°C/min to 290°C and maintained for 39 min. The injected volume was 5 µL with a split ratio 40:1. Compound identification was performed by comparing retention times (RT) and mass spectra with the NIST 2008 and Wiley 2008 libraries. Compound percentages were calculated based on peak areas obtained from the MS data.

2.3. Anti-diabetic Activity Assay

2.3.1. *Determining α-amylase inhibitory activity*

α-Amylase inhibitory activity of Aeo, Pho, and Aao was tested spectroscopically with slight modifications to the method presented by Quan *et al*. (2019). In brief, 25 µL of sample solution at different concentrations and 50 µL of α-amylase solution (0.1 U/mL) in a phosphate buffer (prepared with 6 mM NaCl at a pH of 6.9, 20 mM phosphate buffer) were mixed in a 96-well microplate. The mixture was pre-incubated for 10 min at 37°C. After pre-incubation, 50 µL of starch solution (0.05%) was added and incubated for 10 minutes at 37 °C. The reaction was stopped by adding 25 µL of HCl (0.1 M), and then 100 µL of Lugol solution was added for monitoring. A 96-well microplate reader was used to measure the absorbance at 565 nm.

2.3.2. *Determining α-glucosidase inhibitory activity*

α-Glucosidase inhibitory activity of Aeo, Pho, and Aao was determined using a spectroscopic method with slight modifications (Kim *et al*., 2000). In short, 50 µL of phosphate buffer (at pH 6.9; 10 mM), 25 µL of PNPG (*p*-nitrophenyl-α-*D*-glucopyranoside) in a phosphate buffer (at pH 6.9; 10 mM), 10 µL of the sample solution, and 25 µL of α-glucosidase (0.1 U/mL) in a phosphate buffer (at pH 6.0; 10 mM) were mixed in a 96-well microplate. After 20 min of

incubation at 37 °C, 90 µL of sodium carbonate (100 mM) was added to each well to stop the enzymatic reaction. The absorbance was recorded at 400 nm using a 96-well microplate reader.

2.4. Statistical Analysis

The data of all biological activities, however, were given as average of three parallel measurements, respectively. All biological activity assays were carried out at four different concentrations, and the results were presented as IC_{50} values. Data were recorded as mean ± SEM (standard error of the mean) $p<0.01$.

3. RESULTS

3.1. Fatty Acid Compositions

Comparison of fatty acid composition (FAME) results of Aeo, Pho, and Aao are given in Table 1. In the fatty acid content of Aeo, a total of 16 fatty acids have been identified, with linoleic acid (41.03%), palmitic acid (27.96%), and oleic acid (20.58%) as major components. It has been determined to contain 34.44% saturated fatty acids, 24.53% monounsaturated fatty acids, and 41.03% polyunsaturated fatty acids. Omega 6 with linoleic acid (41.03%); omega 7 with palmitoleic acid (0.55%) and *cis*-10 heptadecenoic acid (0.31%); omega 9 with oleic acid (20.58%), elaidic acid (1.35%), *cis*-10-nonadecenoic acid (1.58%), and ricinoleic acid (0.16%) were included.

In the content of Pho, a total of 18 fatty acids have been identified, with linoleic acid (57.15%), oleic acid (25.16%), and palmitic acid (7.58%) as major components. It has been determined that 14.62% are saturated fatty acids, 28.23% are monounsaturated fatty acids, and 57.15% are polyunsaturated fatty acids. Omega 6 with linoleic acid (57.15%), omega 7 with palmitoleic acid (0.19%) and *cis*-10 heptadecenoic acid (0.11%), omega 9 with hypogeic acid (0.09%), oleic acid (25.16%), elaidic acid (2.05%), *cis*-10-nonadecenoic acid (0.16%), and gondoic acid (0.47%) were founded.

In the fatty acid content of Aao, a total of 12 fatty acids have been identified, with oleic acid (64.44%), linoleic acid (14.21%), and palmitic acid (12.18%) being the major components. It has been determined to contain 16.65% saturated fatty acids, 69.14% monounsaturated fatty acids, and 14.21% polyunsaturated fatty acids. Omega 6 with linoleic acid (14.21%), omega 7 with palmitoleic acid (0.64%) and *cis*-10 heptadecenoic acid (0.17%), omega 9 with hypogeic acid (0.12%), oleic acid (64.44%), elaidic acid (2.93%), and gondoic acid (0.43%) were included.

3.2. Anti-diabetic Activity

A comparison of the anti-diabetes activity results of Aeo, Pho, and Aao is given in Table 2. According to the anti-diabetic activity, in the α-amylase inhibition assay, the IC_{50} values of three oils were less than 50 µg/mL. Pho (IC_{50}: 15.36 ± 0.52 µg/mL) and Aao (IC_{50}: 7.38 ± 0.47 µg/mL) were found to be more active than acarbose (IC_{50}: 23.40 ± 0.26 µg/mL), which is the positive standard of the test. In the α-glucosidase inhibition test, all tested oils showed greater activity than acarbose (IC_{50}: 304.36 ± 1.97 µg/mL). Among them, the most active oil, Aao (IC_{50}: 60.40 ± 2.38 µg/mL), was five times more effective than acarbose, while Pho (IC_{50}: 170.29 ± 2.14 µg/mL) exhibited approximately two times better activity than acarbose.

Table 1. Comparison of fatty acid composition (FAME) results of *A. esculentus* (Aeo) and *P. harmala* (Pho) seed oils with *A. agallocha* (Aao) maceration oil.

GC-MS Fatty Acid Compositions							
Peak	RT (min)	Fatty acid (IUPAC Name)	Special Name	Lipid Profile	Aeo (%)	Pho (%)	Aao (%)
1	16.361	Tetradecanoic acid	Myristic acid	C14:0	0.21	0.10	-
2	19.683	Pentadecanoic acid	Pentadecylic acid	C15:0	0.02	0.83	-
3	21.936	7-Hexadecenoic acid	Hypogeic acid	C16:1	-	0.09	0.12
4	22.091	(9*Z*)-9-Hexadecenoic acid	Palmitoleic acid	C16:1	0.55	0.19	0.64
5	23.018	Hexadecanoic acid	Palmitic acid	C16:0	27.96	7.58	12.18
6	25.228	*cis*-10 Heptadecenoic acid	*cis*-10 Heptadecenoic acid	C17:1	0.31	0.11	0.17
7	26.169	Heptadecanoic acid	Margaric acid	C17:0	0.16	0.10	0.10
8	28.216	(9*Z*, 12*Z*)-Octadeca-9,12-dienoic acid	Linoleic acid	C18:2	41.03	57.15	14.21
9	28.472	(9*Z*)-Octadec-9-enoic acid	Oleic acid	C18:1 cis-9	20.58	25.16	64.44
10	28.562	(9*E*)-Octadec-9-enoic acid	Elaidic acid	C18:1 trans-9	1.35	2.05	2.94
11	29.314	Octadecanoic acid	Stearic acid	C18:0	4.89	3.90	3.62
12	30.177	*cis*-10-Nonadecenoic acid	*cis*-10-Nonadecenoic acid	C19:1	1.58	0.16	0.40
13	33.411	12-Hydroxy-9-Octadecenoic acid	Ricinoleic acid	C18:1	0.16	-	-
14	34.202	*cis*-11-Eicosenoic acid	Gondoic acid	C20:1 cis-9	-	0.47	0.43
15	35.138	Eicosanoic acid	Arachidic acid	C20:0	0.70	1.08	0.63
16	37.910	Heneicosanoic acid	Heneicosylic acid	C21:0	-	0.04	-
17	40.576	Docosanoic acid	Behenic acid	C22:0	0.33	0.69	0.12
18	43.148	Tricosanoic acid	Tricosylic acid	C23:0	0.04	0.12	-
19	45.632	Tetracosanoic acid	Lignoceric acid	C24:0	0.13	0.18	-
		Total amount of saturated fatty acids			34.44	14.62	16.65
		Total amount of monosaturated fatty acids			24.53	28.23	69.14
		Total amount of polyunsaturated fatty acids			41.03	57.15	14.21

Table 2. Comparison of anti-diabetic activity results of *A. esculentus* (Aeo), *P. harmala* (Pho), and *A. agallocha* (Aao) oils.

Sample	IC_{50} (µg/mL)	
	α-Amylase inhibitory assay	α-Glucosidase inhibitory assay
Aeo	36.19 ± 1.03	194.33 ± 1.89
Pho	15.36 ± 0.52	170.29 ± 2.14
Aao	7.38 ± 0.47	60.40 ± 2.38
Acarbose*	23.40 ± 0.26	304.36 ± 1.97

***** Values expressed herein are mean ± SEM of three parallel measurements (p<0.05).
***** Reference compounds.

4. DISCUSSION and CONCLUSION

In this study, the oils from the seeds of *A. esculentus* and *P. harmala* grown in different regions of Muğla province were obtained by the cold-pressing technique, while *A. agallocha* oil was prepared by the maceration technique. The oils were analyzed by Gas Chromatography-Mass Spectrometry (GC/MS) to determine their fatty acid compositions. Fatty acids were screened, and it was determined that palmitic acid ($C_{16:0}$), linoleic acid ($C_{18:2}$), and oleic acid ($C_{18:1}$ *cis*-9) were the major components in all three species. Based on the fatty acid content of internal pharmaceuticals containing oils and externally used oils, preparations suitable for different skin types can be formulated. Oils containing saturated fats are suitable for sensitive skins, those with omega 3 are recommended for acne-prone and blemished skins, omega 5-containing oils are utilized for lightening spots and anti-aging, omega 6 containing oils are beneficial for combination skins, and omega 9-containing oils, owing to their ability to penetrate dry skin, can be included in the formulation of cosmetic and aromatherapy preparations. In this context, considering the fatty acid content of the oils analyzed, three oils contain saturated fats. Thus, oils with high omega 6 content, like Aeo and Pho, are suitable for combination skin types, while Aao, containing high levels of omega 9, is considered suitable for preparations intended for dry skin. Aeo, the values of linoleic acid range from 23.6% to 50.65%, while palmitic acid varies between 10.3% and 36.35% (Jarret, *et al*., 2011). In Pho, a total of 17 fatty acid components have been identified, with linoleic acid being the highest at 61.46% (Kaya & Akbas, 2023). It has been found that among the three types of oils, Aao exhibited superior inhibition against both α-amylase and α-glucosidase compared to acarbose. Aleissa *et al.* (2022) reported that the mucilage and extract of *A. esculentus*, traditionally used in the treatment of type 1 diabetes in Traditional Chinese Medicine, demonstrated a significantly advantageous diabetic effect when a single dose of STZ was administered into the peritoneum of Wistar rats compared to okra seed crude extract. The *A. esculentus* extract exhibits anti-diabetic activity, contributing to a general decrease in blood sugar levels. Based on the activity results, they reported that a solid oral formulation could be developed using okra mucilage crude extract and aqueous seed extract obtained from okra as a pharmaceutical excipient (Aleissa *et al*., 2022). The ethanolic extract of *P. harmala* seeds clearly demonstrated a significant reduction in blood sugar levels at doses of 150 and 250 mg/kg in both normal and diabetic rats (Singh *et al*., 2008). They explained that the ethanolic extract of *A. agallocha* flowers effectively inhibited both α-amylase and α-glucosidase enzymes *in vitro* in a dose-dependent manner (Rajagopal *et al*., 2016). The most reactive regions in fatty acids are the carboxyl group and double bonds (Scrimgeour *et al*., 2005). This may be because Pho (85.38% unsaturated fatty acids) and Aao (83.35% unsaturated fatty acids) inhibit both enzymes in their anti-diabetes inhibition activity. Aao holds promising potential as a natural resource for addressing one of today's most significant issues, type-2 diabetes.

Acknowledgments

A part of this work was previously presented at the 12th Aegean Analytical Chemistry Days.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Yunus Çetintaş: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing - original draft, Methodology, Supervision, and Validation. **Ayşe Çetintaş**: Methodology, Software, Formal Analysis. **Yusuf Sıcak**: Methodology, Software, Formal Analysis. **Mehmet Öztürk**: Methodology, Software, Formal Analysis.

Orcid

Yunus Çetintaş https://orcid.org/0000-0001-6707-6112
Ayşe Çetintaş https://orcid.org/0000-0003-1347-278X
Yusuf Sıcak https://orcid.org/0000-0003-2339-5837
Mehmet Öztürk https://orcid.org/0000-0001-8932-4535

REFERENCES

Alam, J., Mujahid, M., Rahman, M.A., Akhtar, J., Khalid, M., Jahan, Y., ..., & Iqbal, S.S. (2015). An insight of pharmacognostic study and phytopharmacology of *Aquilaria agallocha*. *Journal of Applied Pharmaceutical Science, 5*(8),173-181. https://doi.org/10.7324/JAPS.2015.50827

Aleissa, M.S., Mohammed, A.Z., Alneghery, L.M., Hasnain, M.S., Almutairi, B., Ali, D., Alarifi, S., & Alkahtani, S. (2022). Comparative study of the anti-diabetic effect of mucilage and seed extract of *Abelmoschus esculentus* against streptozotocin-induced diabetes in rat model. *Journal of King Saud University-Science, 34*(8), 102297. https://doi.org/10.1016/j.jksus.2022.102297

AOCS (2007). Free Fatty Acids in Crude and Refined Fats and Oils, Revised 2017, Official Method Ca 5a-40.

Baysal, A. 2011. *Trans yağ asitleri ile insulin direnci ve diyabet etkileşimi [Interaction of trans fatty acids with insulin resistance and diabetes]*. *Beslenme ve Diyet Dergisi*, *39*(1-2), 5-8. https://doi.org/10.33076/2011.BDD.334

Dubey, P., & Mishra, S. (2017). A review on: Diabetes and okra (*Abelmoschus esculentus*). *Journal of Medicinal Plants Studies, 5*(3), 23-26.

Gazem, R.A.A., & Chandrashekariah, S.A. (2014). Omega fatty acids in health and disease: a review, *Journal of Pharmacy Research*, *8*(8), 1027-44.

Jarret, R.L., Wang, M.L., & Levy, I.J. (2011). Seed oil and fatty acid content in okra (*Abelmoschus esculentus*) and related species. *Journal of Agricultural and Food Chemistry*, *59*(8), 4019-4024. https://doi.org/10.1021/jf104590u

Kaya, E., & Akbas, P. (2023). Determination of the antimicrobial, antioxidant activities and fatty acid composition of *Peganum harmala* Seed. *Brazilian Archives of Biology and Technology*, *66*, e23220237. https://doi.org/10.1590/1678-4324-2023220237

Kim, J.S., Kwon, C.S., & Son, K.H. (2000). Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. *Bioscience, Biotechnology and Biochemistry, 64*(11),2458-2461. https://doi.org/10.1271/bbb.64.2458

Kıvrak, I., Kivrak, S., & Karababa, E. (2020). Assessment of bioactive compounds and antioxidant activity of Turkey tail medicinal mushroom *Trametes versicolor* (Agaricomycetes). *International Journal of Medicinal Mushrooms*, *22*(6), 559-571. https://doi.org/10.1615/IntJMedMushrooms.2020035027

Komeili, G., Hashemi, M., & Bameri-Niafar, M. (2016). Evaluation of anti-diabetic and antihyperlipidemic effects of *Peganum harmala* seeds in diabetic rats. *Cholesterol*, Article ID 7389864. https://doi.org/10.1155/2016/7389864

Lopez, S., Bermudez, B., Ortega, A., Varela, L.M., Pacheco, Y.M., Villar, J., Abia, R. & Muriana, F.J. (2011). Effects of meals rich in either monounsaturated or saturated fat on lipid concentrations and on insulin secretion and action in subjects with high fasting triglyceride concentrations. *The American Journal of Clinical Nutrition*, *93*, 494-499.

Pranakhon, R., Pannangpetch, P., & Aromdee, C. (2011). Anti-hyperglycemic activity of agarwood leaf extracts in STZ-induced diabetic rats and glucose uptake enhancement activity in rat adipocytes. *Songklanakarin Journal of Science and Technology*, *33*(4), 405-410.

Quan, N.V., Xuan, T.D., Tran, H.D., Thuy, N.T.D., Trang, L.T., Huong, C.T., Andriana, Y., & Tuyen, P.T. (2019). Antioxidant, α-amylase and α-glucosidase inhibitory activities and potential constituents of *Canarium tramdenum* bark. *Molecules*, *24*(3),605. https://doi.org/10.3390/molecules24030605

Rajagopal, P.L., Premaletha, K., & Sreejith, K.R. (2016). Antidiabetic potential of the flowers of *Aquilaria agallocha* Roxb. *World Wide Journal of Multidisciplinary Research and Development*, *2*(4), 22-24.

Scrimgeour, C. (2005). *Bailey's Industrial Oil and Fat Products*, Sixth Edition, Six Volume Set, Chemistry of Fatty Acids, Edited by Fereidoon Shahidi. John Wiley and Sons. 1-5.

Singh, A.B., Chaturvedi, J.P., Narender, T., & Srivastava, A.K. (2008). Preliminary studied on the hypoglycemic effect of *Peganum harmala* seeds ethanol extract on normal and streptozocine induced diabetic rats. *Indian Journal of Clinical Biochemistry*, 23(4), 391-393. https://doi.org/10.1007/s12291-008-0086-3