SHORT COMMUNICATIONS

APPLICATION OF VEGETABLE OILS AS NATURAL, GREEN AND SUSTAINABLE SOLVENTS FOR EXTRACTION OF PLANT MATERIALS: STUDY OF PHYTOCHEMICAL CHARACTERIZATION AND CHEMICAL PROFILING OF VARIOUS OLEO-EXTRACTS OF *GLYCYRRHIZA GLABRA*

ABSTRACT

In the last decade there is a growing interest in application of green and more friendly environment solvents in both industrial and academia sectors due to various environmental concerns. Vegetable oils has been used as effective natural non-toxic and environment-friendly solvents for extraction of various classes of phytochemical constituents from different herbs. In the present study, various edible vegetable oils like palm oil, rice bran oil, sesame oil and sunflower oil were used for preparation of oleo-extract of a medicinal plant *Glycyrrhiza glabra,* commonly known as Licorice. The resulting extracts were analyzed by HPTLC. Determination of Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC) was carried out by UV-Vis spectrophotometry method for standardization of the oleo-extracts of the herb. The HPTLC fingerprint showed presence of licorice components and phenolics and flavonoids in various oleo-extracts of the herb. So the oils exhibited satisfactory solvent effects with capability of extracting various phytochemicals from licorice and can be a used as a greener, safer and alternative approach to petrochemical solvents for herbal drug extraction and enrichment of phytoconstituents.

Keywords: Edible vegetable oil, green solvent, oleo-extract, herbal drugs, chemical profiling

INTRODUCTION

Herbal extraction is a key process to prepare various active ingredients, for applications like cosmetic, perfume, nutraceutical, food, pharmaceutical and fine chemicals. The conventional extraction process requires large quantity of organic solvents for extraction, followed by concentration of miscella to remove solvent to get the herbal extracts. Both the extraction process as well the concentration of miscella for solvent removal are energy consuming processes that needs a lot of energy[1]. The solvents mostly used are hexane, chloroform, ethyl acetate and methanol. The organic solvents are usually highly volatile, inflammable and highly toxic for environment and human health. The solvents are reported for adverse effects on human health like carcinogenic, mutagenic, reprotoxic and endocrine disruptor. The lipophilic solvents like hexane has been found to solubilizing neural lipids causing harm to human health[2,3]. So, to tackle these issues, there are substantial efforts in the research and development of sustainable solvents as an alternative for green solvents in academia and industry. Though water is one of safest solvents, it is capable of extracting only the highly polar phytochemical constituents while extraction efficiency for lipophilic and medium polar constituents is very low. Some of the other green solvents used in recent times are super critical fluids (water and CO_2), ionic liquids, deep eutectic solvents which are expensive and needs special training for handling[4]. Looking at these concerns, edible vegetables oils can be promising and environment friendly solvent for herbal extraction. The edible vegetable oils are renewable, bio-based, safe, sustainable, non-toxic, non-irritating, recyclable and scalable in nature and offer a cheaper alternative to the petrochemical solvents[5].

Vegetable oils have been successfully reported to extract various range of compounds like volatile terpenoids, essential oils and non-volatile components like phenolics from herbs. For example, soybean oil, sunflower oil, soybean oil, olive oil, palm oil and flaxseed oil have been reported to be used in extraction of phenolics and terpenoids, carotenoids, astaxanthin, flavours, essential oils, antioxidants and pigments from various herbal raw materials like rosemary, oregano, peppermint, garlic, olive leaf, citrus peel, and thyme flower. These extracts are used in food, nutraceutical and cosmetic applications[6,7]. In Ayurveda, different types of lipid-based formulations called as *Tailam* and *Ghritam*, exist that are used for both external and internal application for various ailments. These medicated oils are prepared from herbal materials, milk, vegetable oil e.g. sesame oil, castor oil, coconut oil and ghee in various combinations. In these formulations,

the lipid phase is enriched with phytochemicals that get partitioned from herbal ingredients during the preparation process. Some of the formulations are shadabindu tailam, anu tailam, dashamula tailam, triphala ghritam, jatyadi ghritam and brahmi ghritam[8,9]. Pharmacokinetic study on lipid based formulation has demonstrated increase in the oral bioavailability of poorly soluble drugs, as the drug is presented in GI tract in solubilized format[10]. One such example is increased bioavailability of cannabidol when administered through a sesame oil formulation[11].

In the present study, various edible vegetable oils like sesame oil, sun flower oil, palm oil and rice bran oil were used as natural and green alternative solvent for extraction of the herbal drug *G. glabra,* commonly known as licorice or *Yastimadhu,* using the procedure mentioned in Ayurveda. Further oleo-extract of the herbs was subjected to chemical profiling using HPTLC, TPC, TFC and UV-Vis spectroscopic analysis to check the type of molecules getting extracted into the above vegetable oil from the herbal extract.

MATERIALS AND METHODS

The chemicals such as toluene, ethyl acetate and methanol were of analytical grade. The reagents such as vanillin and anisaldehyde were purchased from Loba Chemie Pvt. Ltd., Mumbai. The vegetable oil used in the extraction process was purchased from the local market.

Extraction of *G. glabra* with vegetable oil

The oleo-extract of *G. glabra* was prepared using the procedure as recommended in the Ayurvedic Pharmacopoeia of India. 500 g of coarse powder of *G. glabra* was extracted with 4 L of water under reflux for 1 h. The water soluble extract was concentrated to 1 L on water bath. Then 500 mL of edible sunflower oil was heated in a stainless steel vessel and the herbal decoction boiled with it, till all the water evaporated to below moisture level less than 2 %. A similar process, is followed to prepare the oleo-extract from other vegetable oils including rice bran oil, palm oil and sesame oil. In this process, the low polar and medium polar phytochemicals are fractionated into the oil phase and this attributes to various pharmacological and therapeutic activity.

Physicochemical parameter evaluation of the vegetable oils

Various basic parameters of the above oils such as Iodine value, saponification value, acid value, density and refractive index were evaluated for all the oils, as per the AOAC methods. Refractive index was determined using Abbe refractometer at 25 °C and the specific gravity was measured by using a 25 mL pycnometer.

Total phenolic content (TPC) determination

Folin-Ciocalteu method at 765 nm was used to determine the TPC of the oleo-extracts. TPC was calculated from calibration curve obtained from gallic acid standard solution having 10-50 mg mL^{-1} concentration range. Content of TPC was expressed as the percentage of gallic acid equivalent (GAE) per gram extract (mg GAE g^{-1} extract).

Total flavonoid content (TFC) determination

Aluminium chloride method was used to determine TFC using UV-visible Shimadzu spectrometer -Model 1700 at 420 nm. Quercetin standard curve using 10 to 50 mg mL^{-1} concentration range was used for quantification of TFC. TFC was expressed as the percentage of quercetin equivalent (QE) per gram of the extract (mg QE/ g extract).

HPTLC fingerprinting and chromatographic condition

10 mL of each of the oleo-extract was sonicated with 5 mL of analytical grade methanol. The methanol fraction was subjected to HPTLC analysis using CAMAG HPTLC system. The sample were applied through Hamilton syringe using CAMAG autosampler-IV, using aluminium precoated silica gel 60F254 HPTLC plates. The TLC was developed using mobile phase toluene, ethyl acetate, formic acid (8:2:0.1; V/V/V) in a saturated twin trough chromatographic glass chamber (CAMAG, Switzerland) and was visualized under UV-chamber (254 and 366 nm) and in visible light after spraying with anisaldehyde sulfuric acid reagent followed by heating at 105 ℃ for 5 minutes till colored zones were visible.

UV-Vis spectrophotometer analysis

For UV-Vis spectrophotometric analysis, methanol soluble fraction of each of the oleo-extracts was scanned in the wavelength ranging from 200 to 800 nm by using Shimadzu spectrophotometer and the characteristic peaks were detected.

RESULTS

Physicochemical analysis of the oils

The physicochemical parameters of all the oils are given below and were as per the reported Codex Standard

of FAO specification. Relative density 40 ℃, Refractive index, saponification value and iodine value for various oils were as follows: sesame oil: 0.916, 1.4651, 188.17, 114.61; sunflower oil:0.819. 1.4613, 191.94 and 123.24; rice-bran oil: 0.918, 1.5142, 183.15 and 101.20 and palm oil: 0.911, 1.4582, 195.25 and 56.18. Among all the oils, sunflower oil was having highest iodine value indicating presence of the highest amount of unsaturated fatty acids.

Total phenolic content and total flavonoid content

The phenolic and flavonoid contents of the herbs attribute to a large part of the bio-activity. TFC of oleo-extract of sesame oil, rice-bran oil, palm-oil and sunflower oil was found to be 46.43, 33.73, 4.13 and 9.73 mg QE g^{-1} of extract, respectively, and TPC was found to be 10.17, 12.93, 48.10 and 20.86 mg GAE g^{-1} of extract. The total phenolic content of hexane extract and methanol extract of *G. glabra* was found to be 71.20 and 4.65 mg GAE g^{-1} of respectively, and total flavonoids of hexane extract and methanol extract *G.glabra* was found to be 9.20 and 830.00 mg QE g^{-1}.

HPTLC chemical profiling of the oil

Chromatography analysis was carried out for chemical fingerprint of all the licorice oleo-extracts prepared using above four vegetable oils using modern HPTLC. The HPTLC chromatogram provided good resolution and led to identification of extracted compounds from licorice. Chromatograms at 254 nm, 366 nm and after derivatization with anisaldehyde and sulphuric acid are showed in Fig. 1 and fingerprint of the extracts are presented in Table I. Among all the oils, sunflower oil and palm oil were having highest capacity to extract more number of components; nevertheless sesame oil and rice-bran oil were also having satisfactory ability in extracting various components from the herb *G. glabra*. Similar pattern was visible after derivatization with vanillin sulphuric acid reagent. Presence of glabridine, a phytochemical marker of *G. glabra,* was confirmed in all the oleo-extracts by comparison with R_f of standard compound.

UV spectroscopic analysis

The UV-VIS spectroscopy is a simple and rapid technique for analysis of various phytochemicals present in the herbal extracts, so it can be used for qualitative analysis of herbal samples. The qualitative UV-Vis profile displayed the compounds separated at various wavelengths at different absorbance for example, SEOEL: 282.50 (1.745), 234.00 (2.416), SUOEL: 277.00 (1.823), 266.00 (3.014), 212.50 (4.000), RBROEL: 279 (1.217), 234 (2.107) and POEL: 276.00 (1.640), 233.50 (2.552), 218.00 (2.052). The number of peaks in the spectrum of oleo extract of various oils were much higher then the corresponding methanolic fractions of the pure oils, indicating presence of various herbal constituents in the oleo-extracts that had got dissolved in the vegetable oils.

Table I: Fingerprint of the oleo-extract and solvent extract of *G. glabra*

Peak	Track					
	1 SEOEL	2 SUOEL	3 RBOEL	4 POEL	5 HEL	6 MEL
1	0.20	0.13	0.13	0.13	0.13	0.11
2	0.25	0.17	0.17	0.17	0.17	0.23
3	0.29	0.23	0.24	0.24	0.29	0.39
4	0.35	0.29	0.29	0.29	0.35	0.49
5	0.46	0.35	0.35	0.31	0.42	0.61
6	0.56	0.42	0.42	0.35	0.46	0.70
7	0.66	0.54	0.50	0.42	0.50	0.79
8	0.77	0.64	0.61	0.54	0.54	0.87
9	0.84	0.70	0.72	0.64	0.64	-
10	0.98	0.79	0.79	0.72	0.67	-
11	-	0.87	0.94	0.79	0.72	-
12	-	0.95	-	-	0.77	-
13	-	-	-	-	0.84	-
14	-	-	-	-	0.87	-
15	-	-	-	-	0.90	-

DISCUSSION

The vegetable oils have been found to be ideal, extraction media of enrichment of various bio-active phytocompounds like anti-oxidants, phenolics, carotenoids, herbal flavours and various essential oils. In additions, the plant based vegetable oils also contains various bio-active molecules such as phytosterols, polyphenols, poly-unsaturated fatty acids, squalene and tocopherol[12]. In the present analysis, various phytochemicals were extracted from the licorice using the edible vegetable based oils. Sunflower and palm oil were found to be having maximum extraction efficiency compared to rice-bran and palm oil in extraction of the phytochemicals. The study showed that vegetable oil has promising solubilizing attributes,

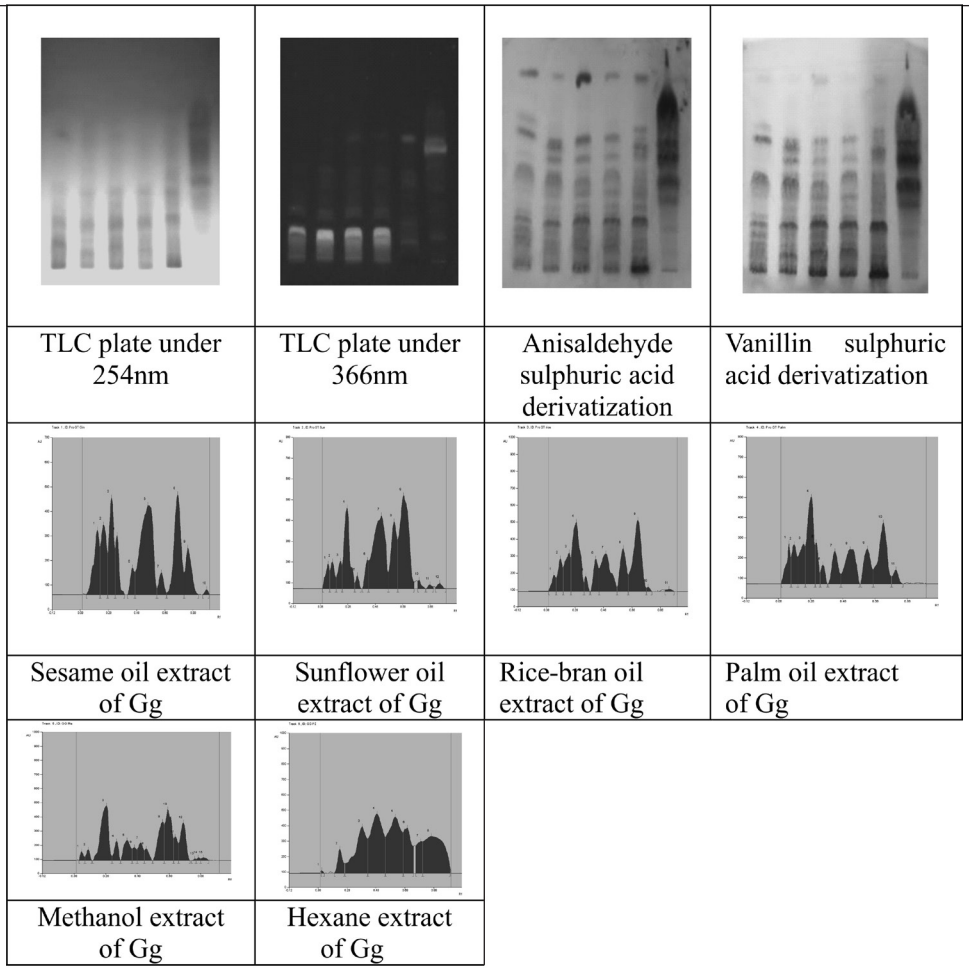

Fig.1: Comparative HPTLC profiling of oleo-extract and solvent extract of licorice. Track 1: SEOEL-Sesame oil extract of licorice, Track 2: SUOEL-Sunflower oil extract licorice, Track 3: RBOEL- Rice bran oil extract of licorice, Track 4: POEL-Palm oil extract of licorice, Track 5:MEL-Methanolic Extract of Licorice, Track 6: HEL-Hexane extract of licorice

like conventional organic solvents and selectivity to extract non-polar to polar phytochemicals from the herbal ingredients. So the vegetable oils can be ideal, non-toxic, environmental-friendly, renewable and bio-based green solvents as a substitute for petrochemical solvents such as hexane and petroleum ether for extraction of low polar to medium polar phytoconstituents. As the vegetable oils are having high flash point and are non-volatile, they would be more safer to environment[1]. These vegetable oil based green solvent do not encounter residual limits like petrochemical non-green solvents like hexane due to their better environmental score.

As the vegetable oil based extracts are more formulation compatible, it can be used easily for various food, pharmaceutical and cosmetic applications. Further, more work has to be carried out for validation of the pharmacological effects and details identification of extracted phytochemicals in the oleo-extract.

CONCLUSION

In the current study the vegetable oils were found to be having satisfactory high solvency in successful extraction of various compounds from the plant *G. glabra*. The compounds identified in the various oleo extract include low polar triterpenoids to mid polar small molecules like phenolics and flavonoids. These vegetable oil based green solvents can be ideal for development of safe and innovative products for health care, personal care and consumer based ingredients. This method can be further used for extraction from other plants of various applications.

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[a] Department of Quality Control & Research and Development, AVN Ayurveda Formulation Pvt. Ltd., Madurai- 625 004, Tamilnadu, India

Manas Ranjan Sahoo[a], Ramesh R. Varier[a], Anitha Kumari R.[a], Bala Tirupura Sundari H.[a], Mageswari H.[a], Bala Guru H.[a] and Ramesh K.[a]

***For Correspondence: E-mail: manasranjansahoo09@yahoo.com**

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