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Presence of biogenic and intrinsically derived organic solvents in *Ginger rhizome* oil: A specific reference to supercritical fluid extract of ginger

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Abstract
The importance of clean and solvent-free pharmaceuticals, nutraceuticals and other allied healthcare (food) products are continuously increasing towards meeting the challenges posed by International regulatory and consumer requirements. The issue of residual solvents in healthcare products is well known requiring consideration, and currently, nutraceutical products derived from medicinal and aromatic plants are of great significance. In this study, specific issues and ambiguities associated with the intrinsically present organic solvents, and, related small molecule constituents in Ginger rhizome oil by supercritical fluid extraction of ginger samples have been investigated. Based on the analysis of the data presented in this study and in combination with the available literature, it is concluded that aromatic plants like Ginger rhizome would tend to contain a varied range of organic solvents like alcohols, esters, aliphatic oxides (ethers) and carbonyls (ketones) in its essential oil or crude extracts. An analysis of the biogenic pathway for the formation of the solvents and other small molecule constituents needs further investigation.

Keywords: Ginger rhizome, organic solvents, supercritical fluid extraction

1. Introduction
For pharmacopeia purposes, residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced during the manufacture of drug substances or excipients, or in the preparation of drug products. This also includes the extraction, isolation & purification of compounds from natural sources. These solvents range from polar like Methanol, Ethanol to nonpolar like Ethyl acetate, Hexane etc. Since residual solvents do not provide therapeutic benefit, they should be removed, to the extent possible, to meet ingredient and product specification, although, the residual solvents may not be completely removed by practical manufacturing techniques. Drug products should not contain higher levels of residual solvents than the safety data Guidelines. It is well known that plants contain various solvents which are produced through bio transformation during the growth of the plants. Some of the most common solvents found in plant extract are alcohols, acetone, ethyl acetate, heptane, etc. Solvent and acid fermentations are common techniques for conversion of glucose and fructose to acetone, ethanol and butanol.

Ginger has been known to contain numerous solvents naturally in the rhizome. Studies have shown the presence of solvents in the rhizome which are naturally biosynthesized. The presence of various hydrocarbons like n-heptane, n-octane, cumene, and alcohols like n-propanol, sec-butanol, carbonyls like acetone, esters like ethyl acetate, methyl acetate, etc. have been reported in ginger oil [1]. Liquid CO2 extraction of ginger rhizomes, followed by fractionation showed the presence of toluene and acetone using gas chromatography-mass spectrometry (GC-MS) analysis [2]. Gong *et al* have identified acetone, propanol, butanol and 2-butanol in steam distilled dry and fresh ginger using GC-MS and chemometric approach [3]. These clearly indicate that the Ginger rhizome could be naturally containing small molecule residual solvents such as alcohols, carbonyls, esters, hydrocarbons and other compounds, and, the presence and percentage content of these could be varying in different samples due to various factors. The current study investigates the presence of volatile organic compounds after supercritical fluid extraction of fresh ginger samples, and compared it with commercial sample of ginger oil and Fresh and dried Ginger rhizome.

Methods

Intrinsically present organic solvents analysis in Ginger rhizome

The following protocol was followed for determination of the inherent and intrinsic residual solvent in Ginger rhizome oil samples.

In case study 1(CS1), a fresh sample of Ginger rhizome oil extracted through SCFE was subjected to complete organic solvents analysis. The sample was analyzed for the residual solvents using Head-Space Gas Chromatography – Mass Spectroscopy (GC-MS) technique.

In case study 2 (CS2), Commercial sample of ginger oil was procured from a supplier in Mumbai, and the sample was outsourced to a third party laboratory for a complete analysis by Head-Space GC-MS to have a comparative analysis of each sample for their intrinsically present organic solvents content.

In case study number 3 (CS3), a Fresh and dried Ginger rhizome were procured from local market, and directly subjected to intrinsically present organic solvents analysis using headspace GC-FID.

Results

Ginger rhizome and its intrinsically present organic solvents analysis

The Ginger rhizome was subjected to SCFE extraction, and the Figure 1 shows the SCFE extraction and the extract obtained from the procedure. The extract was then analyzed using Head-Space GC-MS for the detection of intrinsically present organic solvents and compared with the commercially procured sample and the samples from the Local Market.

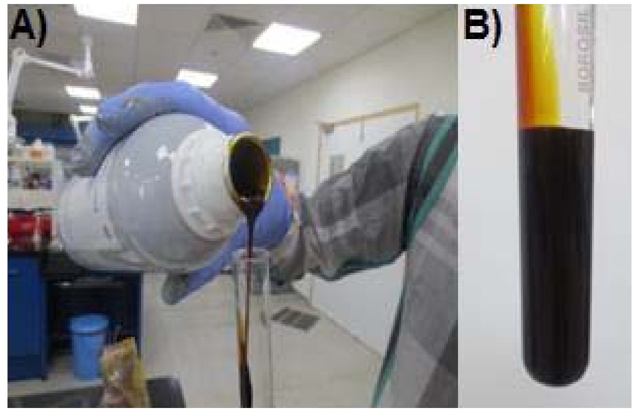

Fig 1: Sample extraction procedure for Ginger *rhizome*: ***panel A*** - Supercritical fluid extract of Ginger sample, ***panel B*** – A thick and viscous sample obtained from the SCFE.

Fig 2: Dried samples of Ginger rhizome collected from the local market

Table 1: shows the comparison of the presence of volatile organic compounds among the samples.

Residual Solvent Detected	Commercial	SCFE sample							
		Batch I	Batch II	LMDG-1	LMDG-2	LMDG-3	LMFG-1	LMFG-2	LMFG-3
Methanol	11.32	108	108	887	330	243	ND	441	231
Ethanol	87.31	38	39	3044	218	302	ND	ND	ND
Acetone	20.23	180	173	1390	701	167	33	82	145
Isopropyl Alcohol	ND	17	8	13	30	15	60	15	31
n-Hexane	ND	14	11	17	18	19	2	2	2
n-Propanol	ND	143	112	23	14	ND	ND	ND	ND
Ethyl Acetate	ND	26	25	125	5	5	ND	ND	ND

Table 1. GC-MS analysis of the residual sample study for the SCFE samples (CS1), Commercial samples (CS2) and samples collected from the local market (CS3). The residue contents of the organic solvents are shown in ppm units. ND indicates – not detected (detection level 1 ppm). LMDG – Local Market Dried Ginger. LMFG Local Market Fresh Ginger.

Discussion

The rhizome of Ginger is one among the aromatic plants that are valued for its by-products more than its fresh or dried plant itself [4]. The extracted ginger oil, irrespective of the extraction process (cold or steam extraction) is known to contain a variety of organic solvents and other small molecule organic constituents. The presence of hydrocarbons, alcohols, carbonyls and esters is well known and has been documented way back in late eighties [5]. It has been recorded that some of the carbonyls and alcohols contribute to the sweet, lemony and flowery flavor of the ginger extract [6]. The presence of citral and citronellyl has also been observed in ginger oil, and it is claimed that their presence is an essential factor contributing to the good odor of the extract [7]. During the storage of the oil or extract sample, some of these organic constituents or solvents (esters, ketones and alcohols) are known to undergo chemical modifications or internal conversions resulting in the formation of a new constituent. Mathew *et al*., have reported that fresh ginger contain a high content of curcumene, and, low concentration of gingiberene in comparison to dry ginger [8]. Salzer *et al*, claimed that the higher concentration of ar-curcumene (curcumene) in fresh ginger oil is due to conversion of gingiberene and beta-sesquiphellandrene in fresh oil [9[]. Even the formation of cinnamaldehyde during the internal conversion is known to bring a sweet and cinnamon flavor to the ginger oil. The thick and viscous nature of the oil with a sweet and cinnamon flavor is an indication of higher content of cinnamaldehyde in the ginger oil.

Plants are known to produce and manufacture a variety of secondary metabolites during their course of growth and survival. They are known to respond to the smallest changes in seasonal variations, and, accordingly their machinery of secondary metabolites production varies with the seasons. Besides a wide range of terpenes and sesquiterpenes, alcohols and esters are the major biogenic metabolites that are produced in measurable quantities. Majority of aromatic plants share this common biogenic pathway in producing same or similar secondary metabolites in the form of esters, alcohols, aldehydes and ketones. The biochemical pathway for the conversion of glucose and fructose to ethanol, acetone and butanol in plants is shown in Figure 3.

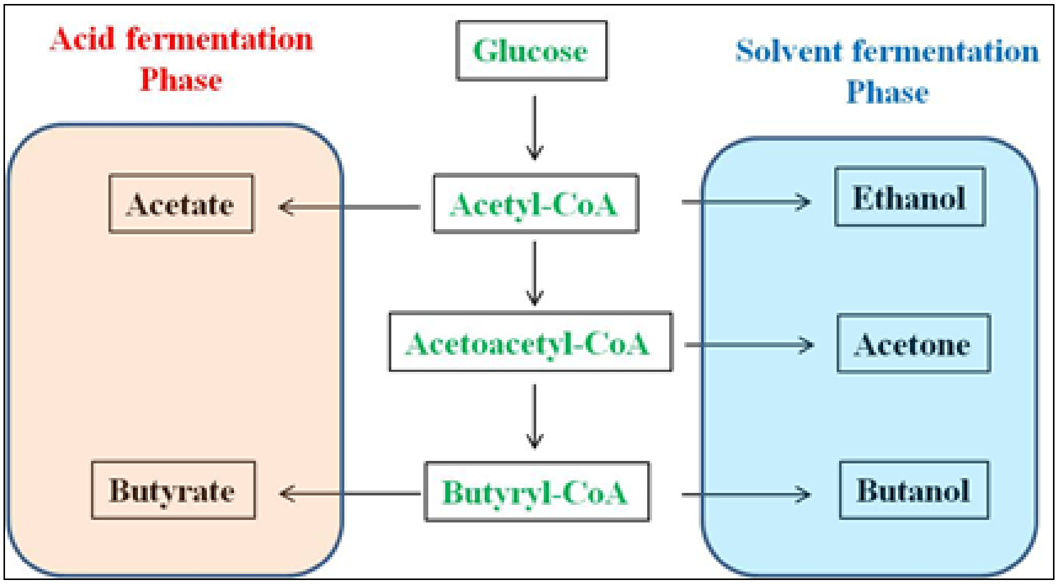

Fig 3: Biogenic or biochemical pathway of conversion of glucose and fructose to ethanol, acetone and butanol in plants.

In conclusion, biogenic by-products of ginger could provide better economic returns to industry if the quality issues could be taken care of. In any case, the organic solvents and constituents are biogenic and inherent in the plant metabolism, and their presence is an added value to the product. A better monitoring of these biogenic by-products would enable the product to reach the prescribed national and international standards.

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