# Chromatographic analysis of essential oils from the leaves of *Fagopyrum esculentum*

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## Abstract

Plant extracts in their crude form or partly purified forms have been employed in the medication for the cure of a number of human diseases since ages. Therefore medicinal herbs are considered to be our precious heritage as these are a part of our traditional as well as modern system of treatment. *Fagopyrum esceulentum* Moench or Buckwheat has a long tradition of being used as food and as medicine because of the abundance of supplements like vitamins, flavonoids, phytosterols ,proteins, polyphenols , minerals and carotenoids, *etc*. in it . Essential oils which may also be referred as Volatile Oils have a distinctive combination of naturally existing compounds and are highly volatile substances in nature . Essential oils (VOS) are present in the form of small droplets in some specific glands of plants. These VOs or essential oils are extracted from various parts of plant like leaves, roots ,stem ,flowers ,seeds etc. The main object of the present work is to separate and investigate different constituents of essential oil from the leaves of the plant *F.esculentum* . The work can be in general categorized in two main parts first is the isolation of essential oil(VO) from the leaves of *F.esculentum* by steam distillation and second is the separation of various components using column chromatographic technique and subsequently identification of various components using GLC .Major constituents identified were α-pinene, β-pinene , Linalyl acetate, d-limonene, p-cymene, Linalool .

Keywords : *F.esculentum*, Essential oils, volatile constituents. Extraction.

## 1. Introduction

In the past few years there has been an increased recognition that more attention needs to be paid for exploring antioxidants and antimicrobial compounds that are present in nature as the interest in the organic products free from any synthetic chemical compound is increasing day by day[1,2] . Recent researches made on secondary metabolites of plants which are considered as a rich source of bioactive compounds have revealed their utility as heptoprotective, anti-diabetic, antimicrobial as well as anti cancerous agents[3].

*Fagopyrum esculentum* Moench.(Common Buckwheat' or 'Kuttu ') is a herb belonging to Polygonaceae family. *F.Esculentum* is an annual herb grown in the Asian region having white or pink flowers and small triangular edible seeds. It is regarded as pseudo cereal as its composition resembles to that of wheat. The buckwheat or kuttu is frequently consumed by human being in the form of flour, sprouts, noodles ,breads and whole seed[4]. *Fagopyrum esculentum* is a very rich source of protein, dietary fibres, amino acids and vitamins .The plant of buckwheat can be effortlessly cultivated in poor soil and can easily bear acidic environment. The main bioactive constituents of *Fagopyrum esculentum* are flavonoids (rutin and quercetin are the main flavanoids[5]) , terpenoids, phenolics and steroids[6].

Essential oils (Volatile constituents ) may be described as odoriferous bodies of oily nature obtained solely from vegetable sources ,which exists generally in liquid state at ordinary temperature and are highly volatile in nature without decomposition. Essential oils or volatile oils are considered as secondary metabolites of plants and are responsible for their odour or aroma .It is well recognized now that the characteristic odour of essential oils is due to the presence of hydrocarbons( terpenes or sesquiterpenes ) and some oxygenated compounds (ethers, alcohols, lactones, phenols and phenolic ethers ) in it[7].

Essential oils which are volatile in nature are present in cell glands, ducts or specific cells located in the plant parts and consists of a distinctive combination of hydrocarbons (terpenes or sesquiterpenes etc.),oxygenated

compounds (aldehydes ,alcohol, ethers , lactones , ketones., phenols etc) and a small percentage of Paraffin wax and resinified products[8,9] . Essential oils play a significant part in the immune system of the plant. Essential oils are collected from various plant parts like stem ,leaves ,seeds, roots , fruits and bark. Plants with essential oils of medicinal importance have been found to possess antimicrobial as well as antioxidant properties[10]. Application of Essential oils in the field Aromatherapy and pharmaceuticals has been well established now .These essential oils or volatile oils are prepared by various techniques such as steam distillation , hydro distillation and solvent extraction technique depending upon the nature of material to be extracted. The common methods used for the extraction of these oils are hydrodistillation method and steam distillation methods. Basically the steam distillation technique allows a single compound or a combination of compounds that are to be refined to pass through a temperature substantially below than the boiling point of the individual constituent present. In steam distillation technique steam is passed in dry form through the plant material and the vapours are finally collected after condensation in a receiver. Although Essential Oil contains components with their boiling points (B.P) up to 200°C or higher temperatures yet in presence of dry steam or boiling water, these constituents can get volatilized at a temperature almost near to 100°C at atmospheric pressure.

Several physicochemical techniques are used to assess the physical properties like refractive index, specific gravity ,optical rotation etc of essential oils[11]. For the investigation of the chemical composition of the essential oils (VOs) chromatographic techniques in combination with various other methods of detection are used frequently.

## 2. Experimental

### 2.1 Plant material and chemicals

The leaves of *Fagopyrum esculentum* were procured from the united chemical and allied products calcutta[12]. All the reagents used in analysis were analytical grade reagents .

### 2.2 Preparation of volatile oils

Separation of essential oil (VOs) from leaves of *Fagopyrum esculentum* was done by steam distillation technique in many sections by using clevenger apparatus . The volume of volatile oils was measured from a calibrated trap. The volatile oils present in the distillate were then dried over anhydrous sodium sulphate ($Na_2SO_4$) and kept in the freezer at a temperature of $4^0$ C in a sealed dark coloured glass bottle until used. The volatile oil was analysed for its physicochemical properties like Saponification value ,acid value ,Specific gravity ,ester value etc.

Volatile oil was found to have following physicochemical parameters

| | | |
|---|---|---|
| 1 | Acid value mg KOH / g oil | 3.120 |
| +2 | Ester value mg KOH / g oil | 42.26 |
| 3 | Saponification value mg KOH / g oil | 95.10 |
| 4 | Specific gravity | 0.9714 |
| 5 | Refractive index | 1.4782 |

### 2.3 Separation of various components of oil by column chromatography

Ethanol extract of the volatile oil of the leaves (about 8.5 gm) was studied by column chromatography over silica gel (100 – 200 mesh – Merck) packed and eluted with various solvents starting with hexane in the increasing order of the solvent polarity hexane, benzene ,chloroform, ethyl acetate and methan.

**Details of column chromatography**

Length of column :16.5 cm

Diameter of column :4.6 mm

weight of silica gel :160gm

weight of the oil :8.5 gm

Eluants from various solvents were then subjected to TLC analysis on glass plates (5x10 cm) coated with a mixture of silica gel (200 mesh) and plaster of Paris. The eluants were spotted 2.5 cm above the bottom of the plate and solvent was used about 8.0 cm from the base line. The plates were then developed .

## 3. Result and discussion

Eluants from hexane were combined and after the removal of solvent were subjected to rechromatography over a column of silica gel and eluted with petroleum ether and a mixture of petroleum ether: Benzene in the ratio of 2:2.On the basis of various physicochemical parameters (refractive index, specific gravity ) two components (α-Pinene and β-pinene) were identified. Similarly eluants of benzene fraction were combined and after removal of solvent were subjected to column chromatography with petroleum ether : benzene (2:2) mixture and on the basis of various physicochemical parameters identification of three compounds(α-thujene, d-Limonene, .Methyl chavicol ) was made. Chloroform eluants showed two spots in TLC and therefore after the removal of solvent it was again chromatographed over a column of silica gel and eluted with chloroform and ethanol and the presence of one compound( 1,8 cineole )was detected on the basis of various physicochemical properties .Ethyl acetate eluant showed two spots in TLC was subjected to column chromatography after the removal of solvent using ethyl acetate and ether (1:1) mixture and one compound (Eugenol) was identified. eluants from methanol gave single spot in TLC and was identified as due to α-terpineol. Results were confirmed by Co-chromatography with authentic samples. Results are given in observation table. .

**Observation table**

| S.No. | Eluants | Number of spots in TLC | Refractive index at $20^0$ C | Specific gravity at $25^0$ C | Compound identified |
|---|---|---|---|---|---|
| 1 | Hexane | Three | a.1.4634<br>b.1.4734<br>c. unidentified | a..8434<br>b..8650 | a. α-Pinene<br>b. β-pinene |
| 2 | Benzene | Three | a.1.4456<br>b.1.4728<br>c.1.515 | a..8286<br>b..8440<br>c. .9320 | a. α-thujene<br>b.d-Limonene<br>c.Methyl chavicol |
| 3 | Chloroform | Two | a.unidentified<br>b.1.4567 | a. unidentified<br>b. .8920 | a.unidentified<br>b.1,8 cineole |
| 4 | Ethyl acetate | Two | a. unidentified<br>b.1.5415 | a. unidentified<br>b.1.052 | a.unidentified<br>b.Eugenol |
| 5 | Metanol | One | 1.4842 | .9345 | α-terpineol |

### GLC Analysis of the volatile oil of leaves of *fagopyrum esculentum*

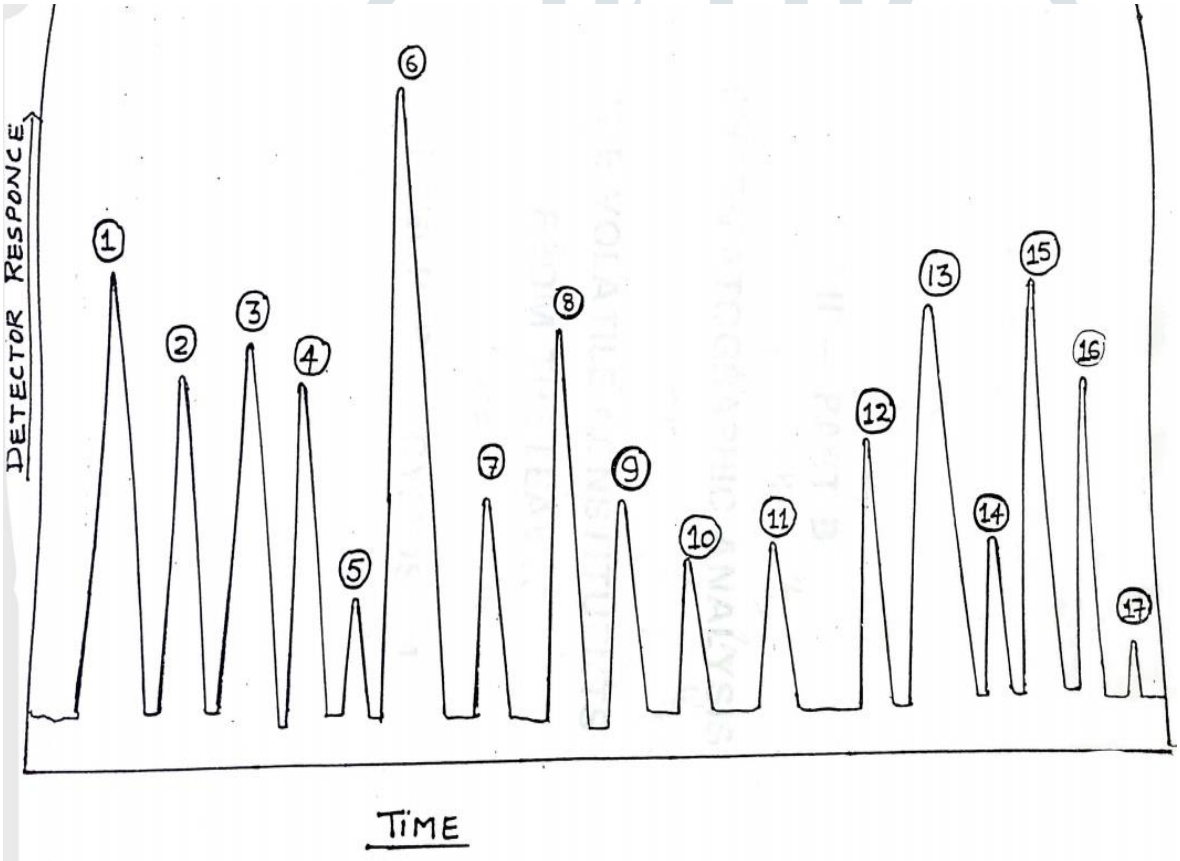
The volatile oil yield ( v/w) from the leaves of *fagopyrum esculentum* after steam distillation was found to be about .55 percent. The volatile constituents of the essential oil of *fagopyrum esculentum* were identified by comparing their retention times at different temperatures with those of the pure components and noting the increase in the areas at the corresponding peaks .Calculation of peak areas was made and used for the quantitative measurements of various components[13].Results are interpreted in table 2 and GLC shown in fig. No.1

**Table :Components of essential oils from the leaves of *fagopyrum esculentum***

| S.No | Compound | Concentration (percentage) | Molecular formula | Molecular weight |
|---|---|---|---|---|
| 1 | α-pinene | 8.20% | $C_{10}H_{16}$ | 136 |

| | | | | |
|---|---|---|---|---|
| 2 | β-pinene | 5.75% | $C_{10}H_{16}$ | 136 |
| 3 | p-cymene | 7.60% | $C_{10}H_{14}$ | 134 |
| 4 | d-limonene | 5.60% | $C_{10}H_{16}$ | 136 |
| 5 | Linalyl acetate | 2.00% | $C_{12}H_{20}O_2$ | 196 |
| 6 | Linalool | 11.8% | $C_{10}H_{18}O$ | 154 |
| 7 | α-Thujene . | 4.6% | $C_{10}H_{16}$ | 136 |
| 8 | α-terpineol | 7.8% | $C_{10}H_{18}O$ | 154 |
| 9 | Bornyl acetate | 4.8% | $C_{12}H_{20}O_2$ | 196 |
| 10 | Myrcene | 3.0% | $C_{10}H_{16}$ | 136 |
| 11 | Camphene | 4.2% | $C_{10}H_{16}$ | 136 |
| 12 | Terpinolene | 5.4% | $C_{10}H_{16}$ | 136 |
| 13 | Methyl chavicol | 8.1% | $C_{10}H_{12}O$ | 148 |
| 14 | Eugenol | 3.60% | $C_{10}H_{12}O_2$ | 164 |
| 15 | α-borneol | 8.9% | $C_{10}H_{18}O$ | 154 |
| 16 | 1,8 -cineole | 6.5% | $C_{10}H_{18}O$ | 154 |
| 17 | Unidentified constituents | .94% | - | - |

Fig No. 1




When the volatile oil from the leaves of *fagopyrum esculentum* was analysed for its chemical composition of by GC-Ms it was found to be abundant in terpenoids (listed in table). From the volatile oil of *fagopyrum esculentum* about 16 compounds were identified which accounted for 97.85% of the total yield. The major components identified in the oil were Linalool (11.8 %), α-borneol (8.9%) , α-pinene (8.2%), Methyl chavicol (8.1%), α-terpineol (7.8%),p-cymene (7.6%),1,8 -cineole (6.5 %) , β-pinene (5.75%), d-limonene , (5.60 %) and Terpinolene(5.4%)[14,15] .

## 4. Conclusion

It is evident from the above studies that the essential oils obtained from the leaves of the plant *fagopyrum esculentum* are rich in phytochemicals which contributes towards the medicinal properties that may be of

potential use as important therapeutic agents. Further investigation on these bioactive compounds can result in the exploration of new drugs for the treatment of various diseases.

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