Fatty acid analysis of *Ocimum tenuiflorum* and *Azadirachta indica* through gas chromatography

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ABSTRACT

In this study we are focusing on the therapeutic uses of major fatty acids of two medicinal plants *Ocimum tenuiflorum* (Tulsi) and *Azadirachta indica* (Neem) by using Gas Chromatography. Oleic acid in Neem and Tulsi is found to be 29.27% and 13.90% respectively, it is the highest percentage of all fatty acids present. Stearic acid was found to be present in maximum percentage in Tulsi (11.50%). It may be further reacted to form stearyl alcohol which is used in industrial and cosmetic products. Heptadecenoic acid was found in both the plants, Cis-9-heptadecenoic acid is used for treating psoriasis and allergies. Thus through the present study we conclude that the fatty acids present in *Ocimum tenuiflorum* and *Azadirachta indica* have medicinal as well as commercial applications.

Keywords: *Ocimum tenuiflorum, Azadirachta indica*, oleic Acid, palmitic acid, gas chromatography

INTRODUCTION

Ayurveda, the ancient Indian therapeutic measure is renowned as one of the major systems of alternative and complimentary medicine. As other herbal systems, greater parts of its medicaments are based on indigenous herbals. And the thorough and fractionate knowledge about the medicinal plants is mandatory for all who are working in the field of ayurveda, in order to identify and select the appropriate plant for a specific disease. In the recent years, the interest in medicinal plants has increased in a great deal. Apart from this, people from the west have also taken this matter seriously by conducting various researches on plant based medicines. Today the large numbers of drugs in use are derived from plants, like morphine from *Papaver somniferum*, aswagandha from *Withania somnifera*, ephedrine from *Ephedra vulgaris*, atropine from *Atropa belladonna*, reserpine from *Roulphia serpentina*, etc. The medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability [1,2].

Imbalances or deficiency of essential fatty acids in the body cause many disorders such as asthma, migraines, inflammations, diabetes, arthritis and other metabolic disorders [3]. In this study we are focusing on the major fatty acid content of two medicinal plants *Ocimum tenuiflorum* and *Azadirachta indica* by using Gas Chromatography and its therapeutic uses.

Modification of fatty acid compositions by metabolic engineering offers good possibilities for producing new oilseed crops with a more balanced α-linolenic/linoleic acid (LA) ratio, preferably combined with high oleic acid (OA) content. In the future, particular attention has to be paid to the proportions of polyunsaturated fatty acids (PUFAs) in the diet, which are the factors that finally

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determine the unique balance of tissue *n*−3 and *n*−6 fatty acids and eicosanoids decisive for human health [4]. It belongs to Lamiaceae family and genus name is *Ocimum* and species is *tenuiflorum* [5]. The leaves contain an essential oil which has been studied with gas chromatography. The oil contains eugenol, eugenal, carvacrol, methyl-chavicol, limatrol and caryophylline and different fatty acid content [6]. Due to the presence of these fatty acids and metabolite it has highly medicinal properties. Essential oil of Tulsi has antibacterial, antifungal and antiviral properties. It inhibits the growth of *E. coli*, *B. anthracis*, *M. tuberculosis*, etc. Its antitubercular activity is one-tenth the potency of streptomycin and one-fourth that of isoniazid [7].

Neem *(Azadirachta indica*) belongs to Meliaceae family. It has also been shown to be effective in controlling foodborne pathogens, making it a potential agent against food spoilage bacteria [8]. Neem oil also contains steroids (campesterol, beta-sitosterol, stigmasterol) and a plethora of triterpenoids of which azadirachtin is the most well known and studied.

MATERIALS AND METHODS

Preparation of plant extract

Twenty five gram of each powdered plant material was soaked with 100ml of 70% methanol (BDH, UK) for 72 hr at room temperature with occasional mixing. Each extract was than filtered with Whatman filter paper number 1, Then filtrate were concentrated in vacuum at 30°C and stored at +4°C until further application. Reagents used were: 0.2 M KOH in methanol: 1.21g KOH in 100ml methanol, hexane, 1 N acetic acid, 1:1, Hexane: Methyl tert-butyl ether (MTBE).

1ml of plant extract was taken in 50ml glass centrifuge tube with teflone-lined cap. 5ml of KOH-Methnol was added to the extract, vortexed well and placed in water bath at 37°C for 1 hour, shaking occasionally. Further 1ml of acetic acid was added and pH was checked with pH paper for neutrality. This was followed by adding 5ml hexane, vortex and centrifuge at 2000rpm for 10 minutes. Top hexane layer was transferred to 15 ml Pyrex tube. The extraction was repeated twice more with 5mls hexane, and then all the hexane layers were combined. Solvent was evaporated under nitrogen just to dryness. And redissolved in 0.8ml of Hexane: MTBE (1:1). Transfer to GC vial and cap. Run on GC.

In GC analysis of the extract of *Ocimum tenuiflorum* and *Azadirachta indica*, capillary columns were used. The columns were installed in 6850 series GC equipped with an injection port with as split ratio of 40:1 and attached to a flame ionization detector (FID). Column temperature was held at 40°C for 4 minutes, and then raised up at 5°C/minutes, to 170°C and held at 300°C for 1 minute. The temperatures of the injector port and detector were set at 230°C. Ultra pure hydrogen gas (99.99 %) was used as carrier gas with a flow rate of 61.5 mL/min and total flow rate is 70.7%. Hydrogen is the carrier gas, nitrogen is the makeup gas and air is used to support the flame. The injected volume of the samples was 2 μL of extra. After separation the fatty acids were identified with the help of MIDI Sherlock software.

RESULTS AND DISCUSSION

In this study methanolic extracts of *Ocimum tenuiflorum* and *Azadirachta indica* were prepared and these extracts were subjected to GC-FAME (gas chromatography) to find out the fatty acid content of the plants. Table 1 shows percentage of fatty acid in *Azadirachta indica* extract and table 2 shows percentage of fatty acid in *Ocimum tenuiflorum* extract. The fatty acid composition was expressed as percentage of total fatty acid methyl ester in the extract [9]. Figure 1 and 2 depict the GC chromatograms of *Ocimum tenuiflorum* and *Azadirachta indica* respectively. The most abundant saturated fatty acids were palmitic (6.1% to 11.0%) and stearic (2.0% to 4.0%). Values from the

literature range from 5.3% to 15.4% for oleic acid, 14.0% to 66.1% for linoleic acid, and 15.7% to 65.0% for linolenic acid [10,14].

Table 1. Percentage of fatty acid in *Azadirachta indica* extract.

Fatty acid	Common name	Percentage (%)
11:0	Undecylic acid	0.11
10:0	Capric acid	3.16
12:0	Lauric acid	2.84
13:0	Tridecylic acid	1.28
14:0	Myristic acid	1.30
15:0	Pentadecylic acid	0.03
15:1	Pentadecenoic acid	0.15
16:0	Palmitic acid	10.27
16:1	Palmitoleic acid	0.48
17:0	Margaric acid	0.46
17:1	Heptadecenoic acid	1.12
18:0	Stearic acid	0.38
18:1	Oleic acid	29.27
18:3	Alpha-linolenic acid	0.15
19:0	Nonadecylic acid	0.59
19:1	Nonadecenoic acid	1.60
20:0	Arachidic acid	0.50
20:1	Gadoleic acid	0.15

Table 2. Percentage of fatty acid in *Ocimum tenuiflorum* extract.

Fatty acid	Common name	Percentage (%)
11:0	Undecylic acid	0.03
10:0	Capric acid	0.32
12:0	Lauric acid	0.52
16:0	Palmitic acid	20.56
16:1	Palmitoleic acid	0.76
17:0	Margaric acid	4.26
17:1	Heptadecenoic acid	5.42
18:0	Stearic acid	11.50
18:1	Oleic acid	13.90
19:0	Nonadecylic acid	0.15
14:1	Myristoleic acid	0.08
20:0	Arachidic acid	0.36
20:1	Gadoleic acid	0.35

In the present study the oleic acid percentage in Neem is 29.27% and it is the highest percentage of all fatty acid present. Whereas in Tulsi oleic acid percentage is 13.90%, this means that oleic acid is major component. Oleic acid is widely used in the industries including textile, chemical, medicine, leather-making, stationary, rolling lubricant, re dressing and paper making etc. As an excipient in pharmaceuticals, oleic acid is used as an emulsifying or solubilizing agent in aerosol products [15]. Oleic acid may hinder the progression of ALD, or adrenoleukodystrophy, a fatal disease that affects the brain and adrenal glands [16]. Oleic acid may be responsible for the hypertensive (blood pressure reducing) effects of olive oil [17].

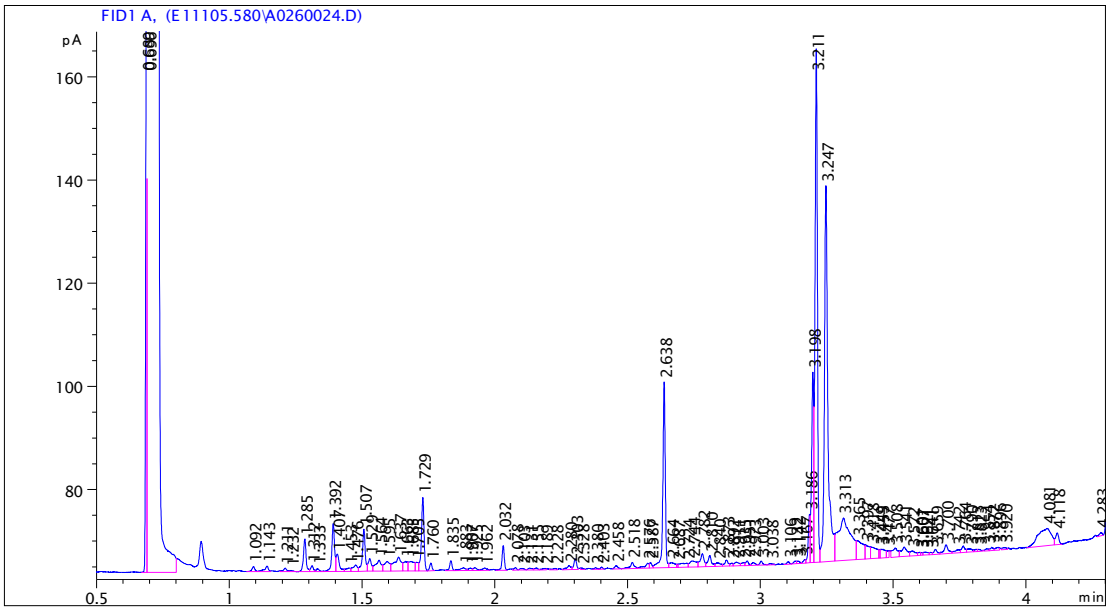

Figure 1. Chromatogram of *Azadirachta indica* extract through Agilent GC 6850 showing the fatty acid peaks.

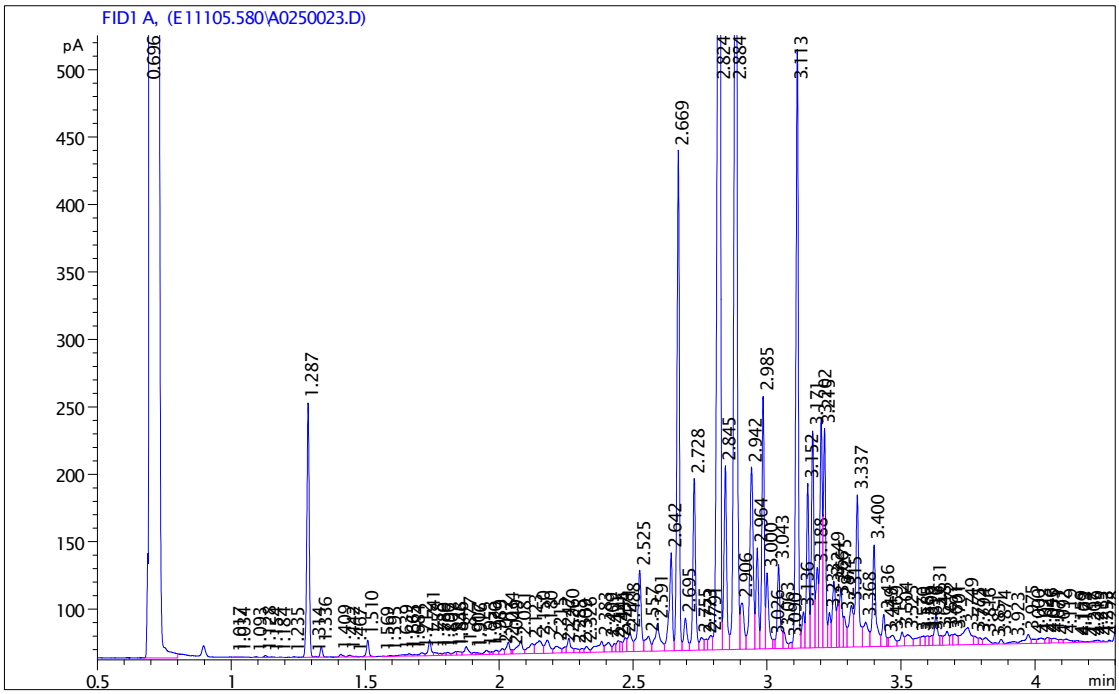

Figure 2. Chromatogram of *Ocimum tenuiflorum* extract through Agilent GC 6850 showing the fatty acid peaks.

Palmitic acid in Neem and Tulsi is found to be 10.27% and 20.56% respectively. It is the second main constituent of whole fatty acid percentage of these plants. Recently, a long-acting antipsychotic medication, paliperidone palmitate (marketed as INVEGA Sustenna), used in the treatment of schizophrenia, has been synthesized using the oily palmitate ester as a long-acting release carrier medium when injected intramuscularly. A study conducted by Hayes et al. showed that when palmitic acid was included in the diet it resulted in decreased cholesterol levels. Stearic acid was found to be present in maximum percentage in Tulsi, i.e. 11.50%. It may be further reacted to form stearyl alcohol which is used in a variety of industrial and cosmetic products. The percentage of margaric acid was found to be more in tulsi, i.e. 4.26%. Other than above described fatty acids these two plants also contain undecylic acid, capric acid, palmitoleic acid, nonadecylic acid, lauric acid, myristoleic acid and gadoleic acid in less percentage. Heptadecenoic acid was found in both tulsi and neem, the percentages are 5.42% and 1.12% respectively. Cis-9-heptadecenoic acid is used for treating psoriasis and allergies. Thus through the present study we conclude that the fatty acids present in *Ocimum tenuiflorum* and *Azadirachta indica* have medicinal as well as commercial applications.

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