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Reaserch Paper

Investigating the possibility of squalene production by some medicinal plants using thin-layer chromatography (TLC)

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Abstract

Medicinal plants contain active ingredients in one or some of their organs. Squalene is one of the active ingredients that prevent heart attacks and cardiovascular diseases and protect the body from some cancers. The aim of the present study was to investigate the presence of squalene in a number of medicinal plants. In this experiment, the plant oils were extracted and measured using Bligh & Dyer with minor changes. TLC (thin layer chromatography) was used to identify squalene. Comparison of TLC of standard squalene with TLC of the investigated medicinal plant samples showed that Caryophillium aromaticus, Descurainia sophia, Portulaca Oleracea, Papaver somniferum and Nigella Sativa contained squalene. Although the percentage of Papaver somniferum and Nigella Sativa seed oil was higher than other medicinal plants, the squalane spot of clove plant had a higher intensity of color and this indicates a higher concentration of squalene in this plant.

Introduction

Squalene is a triterpene with the formula $C_{30}H_{50}$ and an intermediate for the biosynthesis of phytosterol or cholesterol in plants/animals and humans and is common throughout the animal and plant kingdoms. In humans, it appears that newborns have the highest blood squalene concentration, but this suddenly decreases in the opposite direction between the ages of 30 and 40 (Reddy and Couvreur 2009). Due to squalene's significant nutritional benefits, biocompatibility, inertness, and other beneficial properties, squalene is widely used as an excipient in pharmaceutical formulations for the treatment of diseases and therapies. In addition, squalene acts as a protective agent and has been shown to reduce the side effects caused by chemotherapy. In addition, squalene alone exhibits chemopreventive activity. Although it is a weak inhibitor of tumor cell proliferation, it contributes to the treatment of cancer either directly or indirectly due to its potentiation effect (Kumar et al. 2023). In addition, squalene enhances the immune response to various antigens, which is why it is being studied for vaccine delivery applications. Since this triterpene is well absorbed orally, it has been used to enhance oral delivery of therapeutic molecules (Reddy and Couvreur 2009).

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Squalene was first discovered by Japanese scientist Dr. Mitsumaru Tsujimoto. He isolated the non-saponin portion of shark liver oil called kurokozame and discovered the presence of a non-linear unsaturated hydrocarbon (Tsujimoto 1906). Ten years later, Tsujimoto succeeded in recovering unsaturated hydrocarbons with the formula C30H50 from shark liver oil in deep-sea areas, which he named squalene (Tsujimoto 1916). Other researchers (5-8) confirmed the chemical formula proposed by Tsujimoto (Harvey et al. 1926, Heilbron et al. 1926a, Heilbron et al. 1926b, Karrer and Helfenstein 1931). The highest squalene concentration in the world is found in the liver of certain fish species, particularly sharks, which live at depths of less than 400 meters. Research by Tsujimoto showed that squalene is present in at least 16 of 36 shark species in Japanese waters (Tsujimoto 1920). Shark liver oil is the richest source of squalene. However, the limiting factor in using this natural source for squalene production is the presence of persistent organic pollutants and heavy metals, as well as conservation concerns for these marine species (Popa et al. 2015).

In addition to the presence of natural contaminants in squalene extracted from shark liver oil, its use in the pharmaceutical industry is problematic due to the possible presence of various pathogens with which sharks can be infected, as they are transmitted to humans (Popa et al. 2015). In addition, widespread fishing causes many of these species to become extinct because their reproductive cycle is long and their growth is low. For this reason, there is now interest in finding new natural resources for squalene production, especially from herbs (Popa et al. 2015). Squalene is present in many vegetable oils at different concentrations. The first vegetable oil in which squalene was found was olive oil (Thorbjarnarson and Drummond 1935). Frega et al. (Frega et al. 1992) determined the squalene concentration in olive oil to be 564 mg / 100 g, in soybean oil to be 9.9 mg / 100 g, in grape seed oil to be 14.1 mg / 100 g, in hazelnut oil to be 27.9 mgr / 100 g, in peanut oil to be 27.4 mgr / 100 gr, and in corn oil to be 27.4 mgr / 100 gr.4 mgr / 100 gr. The concentration of squalene in vegetable oils produced on a large scale in Europe is 0.19 gr / Kgr in sunflower oil, 0.03 to 0.2 gr / Kgr in soybean oil, 0.1 to 0.17 gr / Kgr in oil corn (Naziri et al. 2011), and 1.7 to 4.6 gr / Kgr in olive oil (Grigoriadou et al. 2007).

Amaranth plants belong to the pseudocereals, which are characterized by excellent nutrient profiles (Venskutonis and Kraujalis 2013). Amaranth seeds, recently introduced in Europe, are now known as the plant with the highest squalene concentration in the plant world, with 4.16 gr / Kgr (León-Camacho et al. 2001). There are about 60 species of Amaranthus, most of which are weeds and some are cultivated that can be used as food, leafy vegetables, fodder and ornamentals (Maurya and Arya 2018). A broader study of 104 genotypes of 30 canopy species showed that squalene concentration varied from 10.4 to 73 gr / Kgr (He and Corke 2003).

Considering the importance of squalane, the aim of this study was to investigate the presence of squalane in some medicinal plants using thin layer chromatography.

Material and Methods

Plant materials

The seeds of clove, opium poppy, soybean, fenugreek, nigella, purslane, fennel, black nightshade, coriander, hemp, flax, and myrtle plants were prepared and powdered.

Oil extraction

Extraction of the oil from the powdered samples was performed according to the extraction protocol of Bligh & Dyer (Smedes and Thomasen 1996). Two replicates were considered for each sample. Briefly, 0.5 g of the seed powder was weighed and placed in a vial. Then, 2 ml of distilled water was added. It was then crushed for 5 minutes using an ultrasonic device. Then 2.5 ml of chloroform and 5 ml of methanol were added, and the homogenizer and ultrasonic device were distributed and rubbed for 4 minutes. 2.5 ml of chloroform and 2.5 ml of water were added again and applied to the homogenizer and vertex for 2 minutes. Then centrifugation was performed at 4000 rpm for 15 minutes, forming two phases. The bottom layer was removed and transferred to a clean vial, then the chloroform solvent was evaporated in the hood. After the solvent evaporated the next day, the remaining oil was mixed with 1-5 / 1 ml of hexane and transferred to the weighed vial, and after the hexane evaporated, the vial was weighed. The weight of the oil was calculated by subtracting the weight of the filled vial from the weight of the empty vial. For the next steps, the vial was covered with parafilm and stored at -20 °C.

TLC analysis:

Thin layer chromatography (TLC) was used to determine the production of squalene in medicinal plant oil (Nakazawa et al. 2014). For TLC, a paper measuring 1.5 * 8.5 cm was cut and the first 1 cm of the paper was marked to determine the location of the oil spot. First, a chloroform-hexane solution (at a ratio of 9-1 ml) is poured into the beaker. One microliter of oil was taken and placed on a TLC paper. The paper with the sprayed oil was placed in the beaker and the lid was covered with parafilm. After 22 minutes, the solution was 1 cm high at the end of the paper and then placed under the hood for 4 minutes to dry. The diluted sulfuric acid was then sprayed on the surface of the paper and placed in an incubator at 80 °C for 50 minutes to better see the separation of the oil components on the paper. The TLC papers were removed from the oven. TLC was also performed with the standard squalene to determine the exact position of squalene on the TLC paper.

Data analysis

The data obtained from the oil percentage were analyzed based on the completely random design. The analysis of variance analysis and the comparison of Duncan's multi-domain mean were performed using Excel and SAS software.

Results

After the oil was extracted from the seeds of the plant samples by Melki et al.'s method, the presence or absence of squalene was confirmed by thin layer chromatography. In this study, pure squalene was used as a standard. The results of thin layer chromatography showed that out of all the samples of medicinal plants, only 5 samples related to clove, poppy, ash, purslane and black seed plants showed the stain related to squalene and the rest of the samples did not show the said spot (Fig. 1).

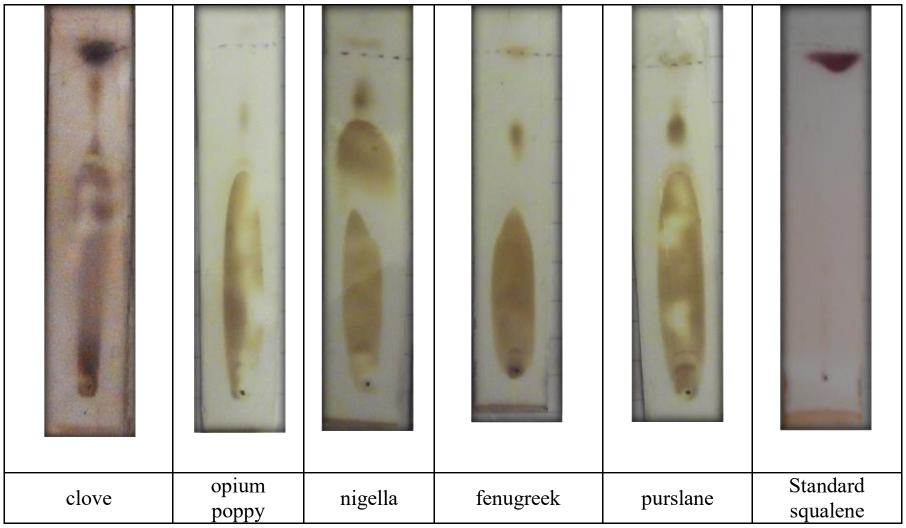

Figure 1- TLC of medicinal plant oil.

Variance analysis of the data obtained from the percentage of oil extracted from 5 plant samples producing squalene showed that there is a significant difference between the amount of extracted oils at the level of 1% (Table 1).

Table 1- Variance analysis of the data obtained from the percentage of oil obtained from some medicinal plants

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Medicinal plants	4	565.3740000	141.3435000	74.06	<.0001
Error	10	19.0850000	1.9085000		

The results of mean comparison using Duncan's multi-range test also showed that purslane and black cumin seed had the highest amount of oil production (Figure 2) and showed that more than 25% of the dry weight of the seeds of these two medicinal plants are oil.

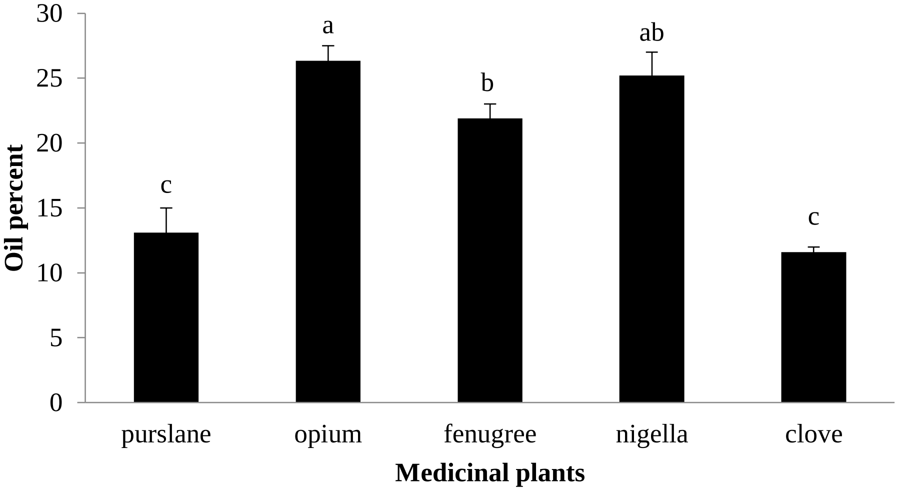

Figure 2. Mean Comparison of the percentage of oil obtained from some medicinal plants

Discussion

Squalene is a triterpenoid compound with multiple applications. This biological molecule acts as an antioxidant, cardioprotectant, anticancer agent, antiviral agent, and skin moisturizer (Ardhyni et al. 2022) and is used in various cosmetic, biomedical, and food industries (Kumar et al. 2023). The diverse and important applications of squalene have increased the global demand for this biological molecule. However, the most important source of squalene production is deep-sea dwelling sharks, the use of which is restricted due to widespread concerns about their extinction. In addition, there is concern about pollution from organic matter and heavy metals. For this reason, extensive efforts have been made to find a suitable alternative. Plants have been proposed as a sustainable alternative for squalene production (Mendes et al. 2022).

So far, various studies have led to the introduction of squalene-producing plants. Calophyllum inophyllum leaves crude n-hexane extract contains 0.009% coumarin, 0.1188% xanthones, 3.9388% friedelin, 11.0262% squalene and 84.9064% other components which friedelin and squalene are the main compounds of this crude extract, where both of them are triterpenes (Ardhyni et al., 2022). Tea leaves also contain squalene. Squalene content Tea leaves grown in the previous year had the highest amount of squalene. Although the amount of squalene in the old leaves of different cultivars was very different, the highest amount of squalene production belonged to the Pingyun cultivar (3.682 mg/g) (Sheng et al., 2022). Soetjipto and Aminu (2022) showed that wild spinach and cockscomb have the relatively high squalene content (Soetjipto and Aminu, 2022).

Quinilla seeds extracts, as a potential source of little-known bioactive compounds with high biological values, contained three main compounds, squalene, phenol, 2,4-bis (1,1-dimethylethyl)-, and n-Hexadecanoic acid (Chañi-Paucar et al. 2023). Also, palm and and Amaranthus oil has been reported to contain high amounts of squalene (Abd Rashid et al. 2023; Maurya and Arya 2018). Although the content of amaranth oil was lower than other oilseeds, however, the squalene content of total lipids was relatively high, ranging from 3.6% in *Amaranthus hypochondriacus* to 6.1% in *A. tricolor* (He et al., 2002).

In our study, the presence of squalene was investigated in 12 medicinal plants, of which 5 medicinal plants, clove, opium poppy, Nigella, fenugreek, and purslane, were identified as squalane-producing plants.

Conclusion

The medicinal plants clove, opium poppy, nigella, fenugreek and purslane are capable of producing squalane. The squalane spot of clove plant had a higher intensity of color, but the amount of oil obtained from the seeds of poppy and black seed plants was higher than the others. It seems that in order to accurately determine the amount of squalene produced by these plants, it is necessary to accurately calculate the amount of squalene produced by these five plants.

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