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ORIGINAL ARTICLE

Facile Synthetical Method of Squalene from Vegetable Residue

H. Ibrahim* **and Ali M. Abubakar**

Department of Chemical Engineering, School of Industrial Engineering, Kaduna Polytechnic, Kaduna-Nigeria.

ABSTRACT – The proscription of the use of shark liver oil for the production of squalene by the International Ocean Conservation and Advocacy Organization (IOCAO) for the protection of sharks in 2005, leads to a great reduction in its quantity in circulation. The quantity in circulation is not enough to meet its growing demand. Therefore, the research for the synthesis of squalene was extended to the use of *Gmelina arborea* leaves. This was carried out by treating the dry leaves of *Gmelina arborea* with methanol and a barium chloride catalyst at 90 °C at varying reaction times in minutes. The products were analyzed using gas chromatography and mass spectrometry (GC-MS). The GC-MS revealed the following results; 162.39, 171.98, 145.69, 92.43, 108.32 and 118.83 mg/g for the reaction times of 10, 20, 30, 40, 50 and 60 minutes, respectively. The maximum yield achieved was 171.98 mg/g at a reaction time of 20 minutes and the minimum yield was 92.43 mg/g at a time of 40 minutes. This method is fast, safe, cost-efficient and economically viable for squalene production. Hence, *Gmelina arborea* has the potential for squalene production to meet up with the global demand.

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INTRODUCTION

Squalene, also known as spinacene or supraene, is a member of the triterpenoid family with six isoprene units [1]. Its I.U.P.A.C. nomenclature is 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene [2]. Squalene ($C_{30}H_{50}$) is a colourless liquid triterpenoid oil that contains 30 carbons and 50 hydrogen atoms [3] as presented in Figure 1. It exists as a linear and also as a coil shape structure [4,5]. It is an isoprenoid, which is an oligomer consisting of six isoprene units that can naturally found in plant sources [1]. It was reported to have been isolated from shark liver oil by a Japanese chemist Mitsumaru Tsujimoto in 1906 [6, 7]. Shark liver oil contains about 80% squalene [1]. It is insoluble in water but miscible in organic solvents such as ethanol, DMSO, and dimethylformamide.

Ancient Japan Shogun recognized it as a source of power, energy, force, and vitality as such they named it Tokubetsu no Miyagi which is translated as a precious gift [8]. It is produced from plants, animals, bacteria, and fungi [9]. It is reported by Huang et al. [10] that squalene has antioxidant, antiperoxidant, and membrane-stabilizing properties. Lou-Bonafonte et al. [11] claim that squalene accumulation in the liver decreases hepatic cholesterol and triglycerides. It has pharmacological, cosmetic, and nutritional potential [8]. It was tested for the treatment of COVID-19 and was found effective as claimed by Ebrahimi et al. [3]. Squalene is translucid, emits a low odour, and offers moisturizing properties, making it one of the most preferred emollients in the world [12]. It is reported by Lou-Bonafonte et al. [11] that squalene has antioxidant, anti-inflammatory, and anti-atherosclerotic properties. In addition to its application as a skin moisturizer, it is also a vaccine or active lipophilic molecule carrier [13] and also an adjuvant in influenza vaccines [14,15].

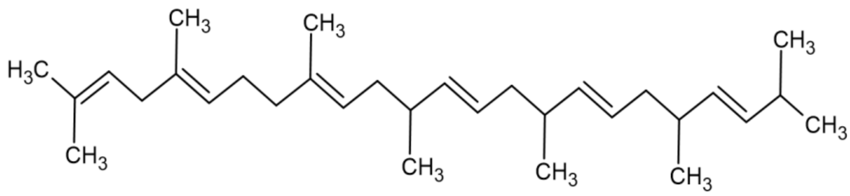
It is used as a precursor for the production of some bioactive compounds in plants such as vitamins, cholesterol, and steroid hormones, as a nutrient, as medicine, inhibits cancer risk and enhanced the antitumor action of chemotherapeutic agents, and improves the immune system of human health [1]. It has antilipidemic, antioxidant, and membrane-stabilizing properties [6]. Its consumption in food prevents cancer and cardiovascular disease [16]. It is also reported by Lozano-Grande et al. [9], that squalene prevents skin damage from ultraviolet radiation. It is very useful for the treatment of diabetes mellitus type 2 and good antitumor [17]. Apart from being an intermediate product in the synthesis of cholesterol, it has many other pharmacological properties such as hypolipidemic, hepatoprotective, cardioprotective, and antioxidant activity [17]. Patil et al. [18] reported that when squalene is applied to the skin it is quickly absorbed deep into the skin keeping the skin healthy.

Patil et al. [18] reported that *Amaranthus cruentus* seed oil contains squalene among other phytochemicals. Revill et al. [19] reported that squalene is produced from deep-sea dogfish (shark) in Australia, New Zealand, India, Northeast Africa, and the Arabian Sea. It is produced by animals and plants but the most commercial source is shark liver oil [20]. Sharks have been the main commercial source of squalene until a restriction was placed by the International Ocean Conservation and Advocacy Organization (IOCAO) for the protection of sharks in 2005 [1]. Senbagalakshmi et al. [1], claimed that shark liver oil contains 80% squalene. Other natural sources of squalene include olive oil, wheat germ oil,

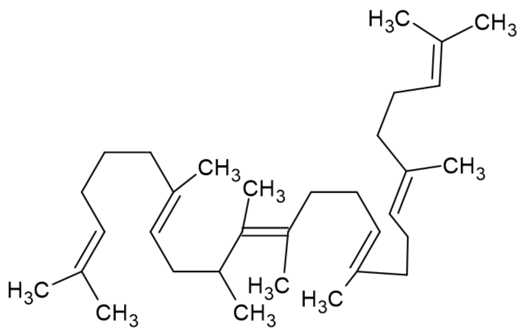
*CORRESPONDING AUTHOR | H. Ibrahim | ibrahimhauna@gmail.com

rice bran oil, amaranth, and palm [8]. It is also found in bacteria, fungi, algae, plants, and animals [21]. Amaranth seed oil is known to be one of the promising sources for commercial squalene production [16].

Martakos et al. [22,23] produced squalene from olive oil. Pokkanta et al. [24], Sugihara et al. [25], and Zullaikah et al. [26] reported the production of squalene from rice bran oil. Palm oil, the most consumable vegetable oil globally is having 200 – 540 mg/kg of squalene [27]. Various techniques have been used to recover squalene from palm oil including microwave-assisted enzyme aqueous which was used by Abd Rasheed et al. [28]. Squalene was found among the co-products of biodiesel in the synthesis of biodiesel from waste *Gmelina arborea* leaves carried out by Magali and Ibrahim [29]. Khatri et al. [30] reported that 0.42% of squalene was obtained from *Gmelina arborea* leaves. The *Gmelina arborea* leaves are abundant in Nigeria. The tree is used to provide shade at residential and institutional premises and the trunk is used for timber production. However, the leaves would fall to the ground and cause a nuisance to the community. Only a small percentage of the fallen leaves serve as feed to domestic animals. The bulk of the leaves will be combusted, thereby polluting the air. Channeling the *Gmelina arborea* leaves into the production of useful products such as squalene is a significant development.



Linear structure



Coil structure

Figure 1. Structural formula of squalene [4,5].

Apart from the work of Magaji and Ibrahim [29] and Khatri et al. [30], there is no known literature on the production of squalene from *Gmelina arborea* leaves. There is no reported work on the effect of reaction time on the yield of squalene from *Gmelina arborea* leaves. This work serves to bridge this gap. In this investigation, squalene was produced from waste *Gmelina arborea* leaves by methanolysis using a barium chloride catalyst at varying reaction times.

MATERIALS AND METHODS

The materials and equipments used in this study include pulverized *Gmelina arborea* dry leaves, distilled water, ceramic mortar and pestle, GallenKamp hot plate magnetic stirrer, thermometer, 1000 mL Pyrex conical flask, 1000 mL Pyrex beaker, separating funnel, and glass funnel. The analytical reagents used were magnesium sulphate, methanol, and barium sulphate.

The dry *Gmelina arborea* leaves were collected within the premises of Kaduna Polytechnic, pretreated by handpicking the dirt, and then pulverized with ceramic mortar and pestle. A solution of 0.5 g of barium chloride catalyst in 500 mL distilled water was prepared in a conical flask. A mass of 50 g of the pulverized leaves was poured into the catalyst solution on a hot plate and heated to 60 °C for 10 minutes at the stirring rate of 250 rpm. The product was first filtered into a 1000 mL beaker with cotton wool and then with filter paper. The filtrate was dehydrated with 0.2% magnesium sulphate in a separating funnel. The lower aqueous layer was tapped off and the upper organic layer was collected and weighed. The experiment was repeated for 20, 30, 40, 50, and 60 minutes. Samples of each product were collected and analyzed with GC-MS to determine the chemical components and their compositions.

EXPERIMENTAL RESULTS

The weights of the filtrates were; 205.09, 179.69, 184.29, 114.09, 132.99, and 151.69 g from the reaction time of 10, 20, 30, 40, 50, and 60 minutes, respectively, as presented in Table 1. The GC-MS analysis revealed the following yields of squalene; 3.96, 4.35, 3.95, 4.05, 4.07, and 3.92%, respectively, as presented in Table 1, and Figure 2. The yield in percentage was converted to weight/weight of feedstock using the expression in Equation 1. The yields (in mg/g) were 162.39, 171.98, 145.69, 92.43, 108.32, and 118.83 mg/g at reaction periods of 10, 20, 30, 40, 50, and 60 minutes, respectively, as presented in Figure 3. A maximum yield of 171.98 mg/g was obtained at a reaction time of 20 minutes and a minimum yield of 92.43 mg/g at a reaction time of 40 minutes as shown in Figure 3.

Table 1. The squalene yield produced from the methanolysis of *Gmelina arborea* leaves.

S/N	Products	Reaction Period (min)					
		10	20	30	40	50	60
1	Filtrate (g)	205.09	179.69	184.29	114.09	132.99	151.69
2	Yield (%)	3.96	4.35	3.95	4.05	4.07	3.92
3	Yield (g)	8.12	8.60	7.28	4.62	5.42	5.94
4	Yield (mg/g)	162.39	171.09	145.69	92.43	108.32	118.83

$$Y = \frac{\%\, yield \times 1000}{wt.of\ filtrate} \tag{1}$$

where Y is the yield in mg/g, % yield is the percentage composition revealed by GC-MS, 1000 is the conversion factor from g to mg, and wt. of filtrate is the weight of the product (in grams) obtained after filtration. The yields of squalene obtained from this study as presented in Figure 2 are higher than 1.26% and 0.42% reported by Magaji and Ibrahim [29] and Khatri et al. [30], respectively.

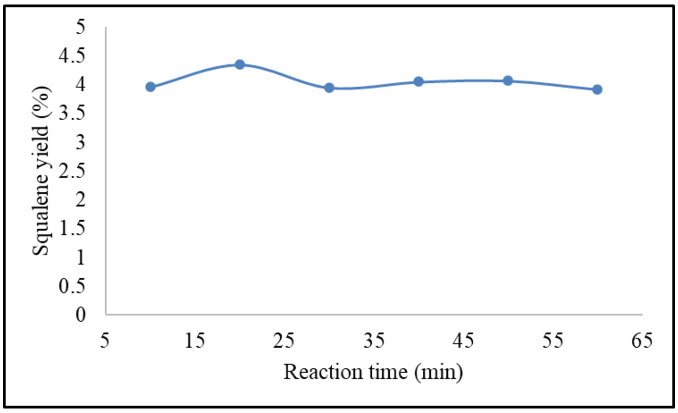


Figure 2. Squalene yields from methanolysis *Gmelina arborea* leaves with time (mins).

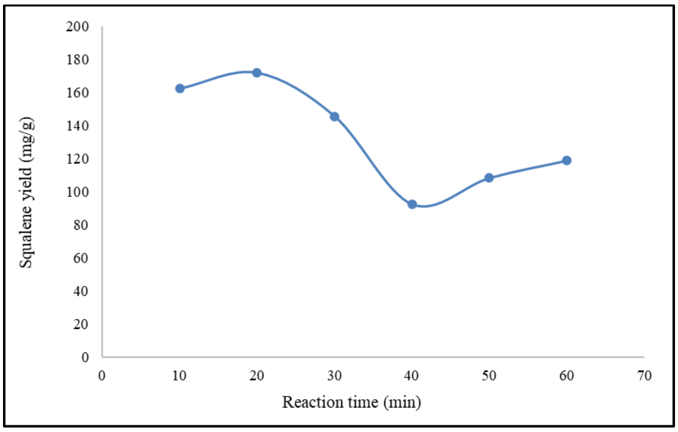


Figure 3. Reaction time for minimum and maximum squalene yields.

The results from this work can be compared with the production of squalene sourced commercially from shark liver oil. However, this source is unsafe due to the risk involved in hunting the fish. Thus comparing the results with *Gmelina arborea* source production would be a better choice. However, there is not much work on the production of squalene from *Gmelina arborea* leaves in literature apart from the works of Magaji and Ibrahim [29] and Khatri et al. [30] as mentioned earlier. The demand for squalene is increasing due to the consumption of natural ingredients in personal care [31]. The effort of Akgun [23] for the production of squalene from olive oil at an optimum temperature of 52 °C, pressure of 0.1 MPa, and extraction time of 3 hours yielded 75%. In another development, up to 250 mg/L of squalene was produced from olive oil by Martakos et al. [22]. The composition of squalene in rice bran oil is estimated to be about 8% as reported by Sugihara et al. [25] as it has a high free fatty acid (FFA) content, waxes, gums, and pigments [26]. Abd Rashid et al. [28] claimed to have recovered 9.61 mg/g of squalene from palm oil using the microwave-assisted enzyme aqueous (MAEA) process. The production of squalene through hydrolysis of fatty acid distillate (FAD) in rice bran oil using lipase Amano, esterify with butanol, distilled, boiled with alcoholic potassium hydroxide and finally extracted with hexane as reported by Nandi and Bhattacharyya [32] is too expensive, too long of a process, and requires too many pieces of equipment. Due to its importance, the current production volume of squalene is far below the global human consumption requirement [9]. The aforementioned methods of squalene production are no doubt more expensive and labour demanding than the use of *Gmelina arborea* waste leaves. This new method, which uses *Gmelina arborea* waste leaves, methanol, and a barium chloride catalyst, is a facile method that will produce squalene in commercial quantities. This new method, using *Gmelina arborea* leaves, will make squalene available to meet the global demand as the tree plantation can easily be established anywhere in the world.

CONCLUSION

Squalene was produced from waste leaves of *Gmelina arborea* by methanolysis method using a barium chloride catalyst at varying reaction times in minutes. The maximum yield of 171.98 mg/g was achieved at 20 minutes and the minimum yield of 92.43 mg/g was obtained at the time of 40 minutes. This method is time-saving, requires fewer materials, saves energy, and is cheaper as the main raw material waste *Gmelina arborea* leaves are costless. Hence, *Gmelina arborea* has the potential for squalene production to meet up with the global demand as it can be cultivated anywhere in the world.

RECOMMENDATION

The effect of reaction temperature on the yield of squalene from *Gmelina arborea* leaves was not considered in this work. There is more room for research in that area. Similarly, the use of other solvents, apart from methanol, could be employed. Other types of underutilized leaves from trees could be explored.

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