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GC-MS Analyses of N-Hexane Extract and Fatty Acids Content in Clerodendrum splendens (Glory Flower) Leaf

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Abstract
The fatty acids contents of the leaves of *Clerodendrum splendens* were analyzed using Gas Chromatography coupled with Mass Spectrophotometer (GC-MS). Fatty acids identified in leaf sample accounted for the ratio of ω-6:ω-3 polyunsaturated fatty acids present as approximately 2:1 and Linoleic acid, an omega-6 fatty acid as having the highest percentage (41.65%) with Capric acid having the lowest percentage (0.0021%). Other fatty acids present as revealed by analysis of n-hexane extract of *C. splendens* are Palmitic (29.33%), Linolenic (16.96%), Oleic (5.03%) and Stearic (3.65%) acids in significant amounts. The study showed that *Clerodendrum splendens* n- hexane extracts could yield polyunsaturated fatty acids (PUFA), the presence of essential fatty acids (EFA) and high omega-6 content in the leaf extract have validated the importance of *C. splendens* in the diet, thus establishing it as pharmaceutically relevant medicinal plant.
Keywords: *Clerodendrum splendens*, fatty acids, GC-MS.

Introduction

Clerodendrum splendens (glory tree) is a species of flowering plant in the genus *Clerodendrum* of the family *Lamiaceae* native to tropical western Africa. It is a twining evergreen climber, growing to 3 meters (9.8 ft.) or more, with panicles of brilliant scarlet flowers in summer. The plant exhibit a wide spectrum of folk and indigenous medical uses mainly for the treatment of asthma and the leaves are traditionally used for the treatment of wounds and burns (Mshana *et al*., 2000), haemorrhoids, diarrhoea and dysentery (Burkhill, 1985). The leaves and roots decoction of *C.splendens* are used to treat tumors, skin disorders, ulcers, abdominal pains, fibroid, asthma, rheumatism and inflammatory diseases (Okwu and Iroabuchi, 2008; Shrivastava and Patel, 2007). Also, the plant is used to treat gonorrhea and syphilis (Okwu and Iroabuchi, 2008).

The validation of the folkloric use of medicinal plant and the analysis of various fatty acids in the plant could reveal how effective it is for treatment of infections, inflammation or life-threatening diseases.

Findings has shown that saturated fatty acid (SFA) intake is associated with coronary heart disease (CHD); increasing SFA intake results in an increase in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) compared to carbohydrate, poly-unsaturated fatty acid (PUFA) and mono-unsaturated fatty acids (MUFA). Individual SFA have differential effects on the lipid profile. Report has shown that lowering dietary SFA to < 7% of energy intake with restricted dietary cholesterol results in further LDL-C lowering than diets containing < 10% of energy intake from SFA. Therefore, replacing SFA with omega-6 PUFA (n-6 PUFA) to achieve a ratio of PUFA to SFA of > 1 will reduce the risk of CHD (National Heart Foundation of Australia, 2009). Likewise, omega-3 fatty acids (ω-3 or n-3) which are also polyunsaturated fatty acids (PUFAs) are important for metabolism (Scorletti *et al*., 2013). The beneficial roles of omega-3 fatty acid supplementation in preventing cardiovascular disease from the perspective of modulating cholesterol had been established (Ibukun and Oladipo, 2016).

One of the most prominent benefits of omega-3 fatty acids is the way in which it averts heart disease. A diet which is rich in monounsaturated and polyunsaturated fats and, at the same time, low in saturated fats will reduce the risk of developing factors associated with heart disease such as high blood pressure and high cholesterol (Hibbeln *et* al., 2006).

Omega-6 fatty acids are a family of pro-inflammatory and anti-inflammatory polyunsaturated fatty acids (Scorletti *et al*., 2013) and its biological effects are largely produced during and after physical activity for the purpose of promoting growth and during the inflammatory cascade to halt cell damage and promote cell repair by their conversion to omega-6 eicosanoids that bind to diverse receptors found in every tissue of the body. The aim of this study is to assess the distribution of omega-6 and omega-3 polyunsaturated fatty acids in the leaves of *C.splendens* and their importance in diets and health.

Materials and Methods

Plant collection

The leaves of *Clerodendrum splendens* were collected in bulk from a Farmland in Oda town, Akure, Ondo State and authenticated at the Department of Crop, Soil and Pest Management, School of Agriculture and Agricultural Technology, Federal University of Technology, Akure. After authentication, the leaves were washed under running tap water to remove adhering dirt followed by rinsing with distilled water, air dried in shadow and

grinded by mixer grinder.

After grinding, 150g of the dried leaf was soaked in 0.6 liters of n-hexane at 40˚C to 45˚C for 6 hours. The organic solvent was filtered through Cheese cloth and whatman filter paper till clear solution was obtained. Solvent was evaporated in a rotatory evaporator (Buchi, Switzerland) under reduced pressure (vacuum) at 40˚C and concentrated. The crude extract was stored in air tight container at dark place.

Fatty acid methyl ester analysis

The lipid extract was used to profile the fatty acids present in the sample using Gas Chromatography and Mass Spectrophotometer. 50mg of the extracted fat content of the extract was saponified (esterified) for five (5) minutes at 95^{O}C with 3.4ml of the 0.5M KOH in dry methanol. The mixture was then neutralized using 0.7M HCl. 3ml of the 14% boron triflouride in methanol was later added. The mixture was heated for 5 minutes at the temperature of 90^{O}C to achieve complete methylation process. The fatty acid methyl esters were extracted thrice from the mixture with redistilled n-hexane. The content was concentrated to 1ml for gas chromatography analysis and 1µl injected into the injection port of GC. The GC equipment used was HP 6890 powered with HP chemstation Rev. A09.01 (1206) software. The split ratio will be 20:1, the carrier gas was nitrogen at inlet temperature of 250^{0}C with a column type of HP INNOWax and column dimensions of 30m x 0.25mm x 0.25µm. The oven program parameters include initial temperature at 60^{0}C, first ramping at 12^{0}C/minutes for 20minutes, maintain for 2minutes and second ramping at 15^{0}C/min for 3minutes, maintained for 8minutes. The detector used FID at 320^{0}C at hydrogen pressure 22psi and compressed air of 35psi.

Results and Discussion

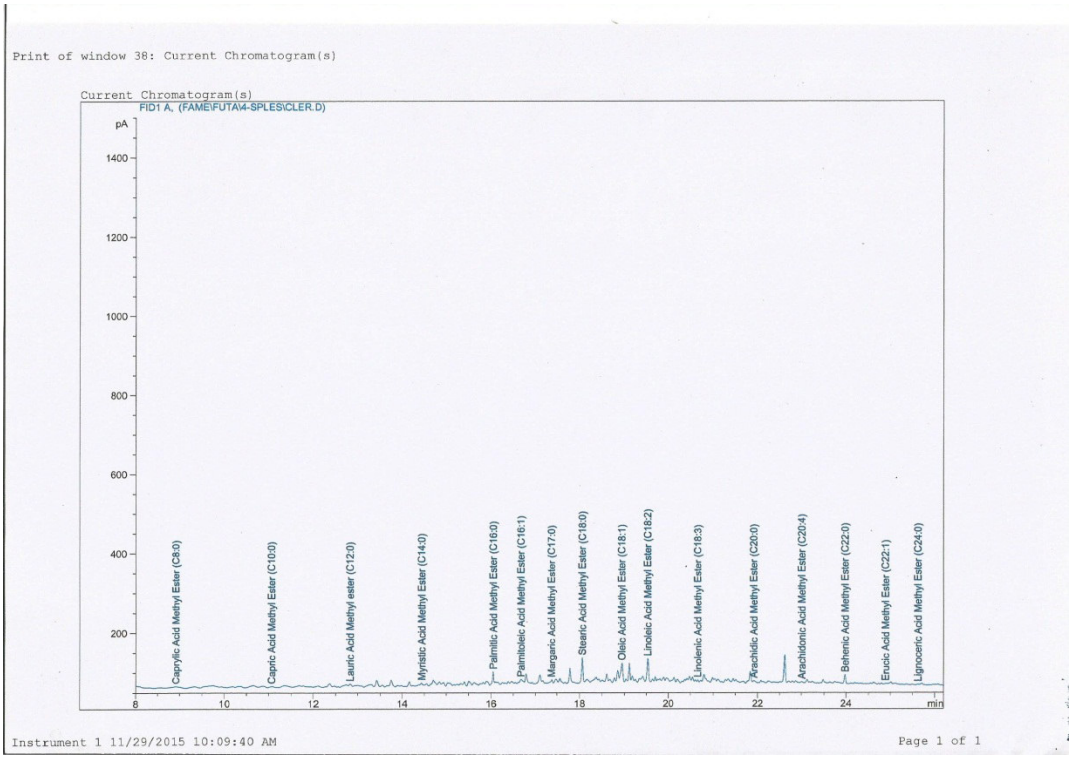

Figure 1: GC-MS chromatogram of n-hexane leaves extract of *Clerodendrum splendens*

Results revealed estimation of the following fatty acids in g/100g of sample as Caprylic acid (C8:0); 0.0026g, Capric acid (C10:0);0.0021g, Lauric acid (C12:0);0.0066g, Myristic acid (C14:0);0.668g, Palmitic acid (C16:0);29.33g, Palmitoleic acid (C16:1); 1.52g, Margaric acid (C17:0);0.039g, Stearic acid (C18:0);3.659g, Oleic acid (C18:1);5.037g, Linoleic acid (C18:2);41.652g, Linolenic acid (C18:3); 16.96g, Arachidonic acid (C20:4);0.044g, Arachidic acid (C20:0); 0.487g , Behenic acid (C22:0); 0.353g, Erucic acid (C22:1); 0.175g and Lignoceric acid (C24:0); 0.053g.

From the GC-MS analysis of *C. splendens* leaves, Linoleic acid has the highest amount compared to the other fatty acids (41.65g/100g of lipid extract) as revealed in the above chromatogram, is an essential omega-6 fatty acid that must be consumed for proper health. A diet only deficient in linoleate, which is the salt form of the acid will cause mild skin scaling, hair loss (Cunnane and Anderson, 1997) and poor wound healing in rats (Ruthig and Meckling-Gill, 1999). Linoleic acid has become increasingly popular in the beauty products industry because of its beneficial properties on the skin. Research points to linoleic acid's anti-inflammatory, acne

reductive, and moisture retentive properties when applied topically on the skin (Darmstadt *et al*., 2002; Letawe *et al*., 1998; Diezel *et al*., 1993).

Oleic acid has a total content of 5.03g /100g of lipid extract from leaves of *C.splendens*. This fatty acid is a common monounsaturated fat in human diet. Monounsaturated fat consumption has been associated with decreased low-density lipoprotein (LDL) cholesterol, and possibly increased high-density lipoprotein (HDL) cholesterol (Nestel *et al.,* 1994). It has been reported that people who are acne-prone tend to have a low percentage of linoleic acid and a high percentage of Oleic acid, but in the sample analysis of the *C. splendens* leaf, linoleic and oleic acids has a ratio of 8:1 and this could be a good indicator that consumption of the leaf could be effective in the treatment of acne (Cardoso *et al.,* 2004), hair loss (Cunnane *et al*., 1997) and poor wound healing in rats (Ruthig *et al* ., 1999).

The combination of Linoleic acid with Palmitic acid has been shown to lower cholesterol levels (French *et al*., 2002). Palmitic acid has a total content of 29.33g/100g of lipid extract of leaves of *C. splendens*. Palmitic acid has been shown to display antioxidant properties and this can help prevent atherosclerosis in rats (Cho *et al*., 2010). This could as well be responsible for the anti-atheroslerotic properties of the leaf. This research revealed that Palmitoleic acid has a total content of 1.52g/100g of lipid extract. Palmitoleic acid is a beneficial fatty acid biosynthesized from Palmitic acid by the action of the enzyme delta-9 desaturase which has been shown to increase insulin sensitivity by suppressing inflammation, as well as inhibit the destruction of insulin-secreting pancreatic beta cells (Yang *et al*., 2011). This might account for the possible anti-inflammatory properties of the leaf.

In epidemiologic and clinical studies, Stearic acid was found to be associated with lowered LDL cholesterol in comparison with other saturated fatty acids (Hunter *et al* ., 2009). Palmitic acid and Stearic acid had been shown to exert a neutral or hypocholesterolemic effect on blood cholesterol levels in experimental animals (Kris-Etherton *et al.,* 2005). Palmitic acid has the second largest percentage in leaf sample (29.33g), while Stearic acid has 3.659g. This may indicate that *C. splendens* leaf has hypocholesterolemic effect on blood cholesterol.

The qualitative and quantitative estimation of fatty acids in the samples revealed the fatty acids using Gas Chromatography Mass Spectrophotometer. The n-hexane extracts contained ω-3 fatty acid (PUFA) precursor, α-linolenic acid (16.96). The ω-6 PUFA present are linoleic acid (41.652) and arachidonic acid (0.044). Report has shown that the verified negligible amount of cholesterol reducing Capric acid is responsible for the normo-cholesterolemic activity (Ibukun *et al*., 2016). Caprylic fatty acids, along Capric and Lauric fatty acids are found in coconut oil. It is a potent antifungal that kills candida cells, as well as restoring acidity of the stomach to its normal levels. Though Caprylic, Capric and Lauric acids contents in leaf sample are (0.0026, 0.0021 and 0.0066 g/100g of lipid extract) respectively, which seems minute in quantity. The presence of these fatty acids suggests the effectiveness of the leaves of *C.splendens* in the reduction of acidic contents in the stomach thereby preventing gastric ulcers as reported by Okwu and Iroabuchi (2008).

Although the overall result revealed that ω-6 PUFAs have a total content of 41.696g per 100g of oil extracts while ω-3 PUFAs have a total content of 16.96g per 100g oil extracts, bringing the ratio of ω-6:ω-3 to approximately 2:1.

Linolenic acid, an omega-3 fatty acid has research showing topical application helps improve many skin concerns, including eczema, psoriasis, acne, and non-melanoma skin cancers (McCusker *et al*., 2010) just like Linoleic acid. It can help repair skin's barrier function and reduce pro-inflammatory substances that would otherwise cause damage. It is believed to communicate with cells that comprise the skin immune system, as evidenced by research showing that topical linolenic and similar fatty acids improve wound healing (Cardoso *et al*., 2004). As preliminary research indicated that ω-3 fatty acids in diets lowered the risk of heart attacks and that ω-6 fatty acids may also reduce the risk of cardiovascular disease (Okamoto *et al*., 2007). The fatty acids composition of the *C. splendens* leaves; and the ω-6:ω-3 ratios can be inferred to be considerably responsible for the anti-atherosclerotic properties, anti-inflammatory activities, importance in health, diet and ameliorative potency in different infectious or life-threatening diseases.

Conclusion

The study had been able to establish *Clerodendrum splendens* leaf as having dietary relevance; the fatty acids composition of n-hexane leaf extract of *C. splendens* as having anti-inflammatory and acne-reducing potential, justified by the amount of omega-6 fatty acids and the ratio of the omega-6: omega-3 values thus establishing the extract as dietary therapy for alleviating inflammation, atherosclerosis and other life-threatening diseases. The ability of the n-hexane leaf extract to ameliorate and bring to normal the cholesterol is characterized by the trace amount of cholesterol reducing Capric acid and Caprylic acid.

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