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Thinlayer Chromatography (TLC) and GC-MS Analysis of Somes Medicinal Plants Used in the Treatent of Haemorrhoids

Eseigbe, M.I.[1*] Salawu, S. O.[2] Adeniyan, O O.[2]
1.Department of Basic Science, Edo State College of Agriculture, Agenebode Campus, Edo State, Nigeria
2.Department of Biochemistry, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria
E-mail: eseigbe.mercy@yahoo.com.

Abstract
Medicinal plant is one in which, one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs The present study aimed at investigating the lipid composition of Axonopus compressus, Anogeiessus leiocarpus and Senna fistula commonly used in the management of haemorrhoids. The lipid analysis of the three plants was determined by TLC and GC-MS and the GC-MS analysis revealed the presence of some saturated fatty acids such as myristic, palmitic, linoleic, stearic, oleic and lauric acid in all the three extracts. In addition A. compressus contained linoleic and palmitoleic acids, S. fistula contained linolenic acid and A. leiocarpus contained linoleic, linolenic and behenic acids. However, palmitic (C_{16}:0)) and stearic acid (C_{18}:0) were observed to be the predominant fatty acids present in all the three plan extracts. The results showed that many active principles are present in the three plants which could contribute to their usefulness in the management of haemorrhoids.
Keywords: Haemorrhoids,Medicinal plants,lipids, TLC,GC-MS

1. Introduction

Various plants were recognized as remedies for some ailments by man through trial and error in the ancient time. Mankind has been exploiting his main nature sources viz: plants, animals and the mineral resources for food, drinks and for the alleviation and prevention of his aliments and diseases in order to sustain life and perpetuate the human species There are records pointing to the fact that herbal medicine has been in practice since a very long time(Elienne, 1988).

Medicinal plants are plants that have medicinal value. Medicinal plants have been a source of succor in the control of many diseases in developing countries and haemorrhoid is no exception (Olumide, 2008). The lower strata of the population living in developing countries rely heavily on medicinal plants due to their cultural alignments as well as their inability to afford the cost of treatment offered by orthodox medical practioners.

In different parts of the world especially in Africa and Europe with high incidence of haemorrhoids, the people have learnt to manage the problem using plants which are God's gift of nature. Crude extracts from plants have been used in treating an array of diseases since ancient time although the bioactive components of such plants remain largely unknown. Various advances in scientific research on the use of plants and herbs brought the beneficial aspects of medicinal plants and the rational for their uses to the lime light (Sofowora, 1993).

All plant contains oil or fats and mainly in their seeds, tubers, bark or root (Eteshola, 2003). The amount of lipids in plant part varies from as low as 0.1% in potatoes to about 70% pecan nuts. Some plants are fat poor (3% in mushrooms), some have middle arrange amount (about 10% in wheat germ), while some are very oily (55% in almonds, 65% in walnuts). Plant lipids has been reported to be used in traditional medicine (Jaffery, 1961) to treat asthma or applied topically to fight inflammation (haemorrhoid). A major component of a safe and effective therapy for haemorrhoids, often overlooked is the use of plant lipids which have been shown to improve haemorrhoids, microcirculation, capillary flow and vascular tone (Dennison *et al*., 1989). The biochemical constituent of plant lipids is proposed to be the fatty acids, essential oils and volatile oils (Mackay, 2001). Fatty acids in plant lipids such as some essential fatty acids cannot be made by the body and must be obtained from diet which help to calm inflammation within the body and lubricate joints (Bloor, 1920) this makes plant lipids a vital component of the human diet. The presence of volatile and essential oils in plant lipids justifies their use in the management of haemorrhoids because they help to fight inflammation (Mark, 1985). It does this by strengthening and improving the vascular structures and make bowel movement easier. The properties bestow high medicinal activities on lipids extracted from plants (Wang *et al*., 1990).

Haemorrhoids or piles are a common ailment among adults. More than half of men and women aged 50 years and older will develop hemorrhoid symptoms during their lifetime. Hemorrhoids are rare in children but now days several reports state the occurrence of haemorrhoids in children, and in elderly people (Madoff *et al*., 2004) Herbal treatments and nutritional therapy are safe and effective therapy for haemorrhoids, although herbal treatments for hemorrhoids have been poorly researched. Several plant extracts have been shown to improve microcirculation, capillary flow, vascular tone, and strengthen connective tissue of the perivascular amorphous substrate. One recent finding showed effects of plants taken orally for treatment of hemorrhoids, is due to the

contribution of free radical scavenging properties, to the pathogenesis of haemorrhoids and varicose veins (Madoff *et al*., 2004). Plants have several properties which make them effective for the treatment of haemorrhoids, like antioxidant, anti-inflammatory, anti-oedema and hepatoprotective. Some plants, which were scientifically studied for their antihaemorrhoidal properties include: *Ruscus aculeatus* (Butcher's Broom), *Collinsonia canadensis* (Stone root), *Aesclus hippocastanum* (Horse Chestnut), *Hamamelis virginiana* (Witch Hazel), *Allium cepa* (Onions), *Averrhoa bilimbi* (Cucumber tree), *Luffa acutangula* Lin.(Luffa), White Oak, Barberry, Ampalaya (Bitter Melon), *S. fistula* (Rangnekar *et al*., 1974) and *A. compressus, A. leicarpus* (Gbededo and Odukoya, 2011).

2. Materials and Methods

2.1 Chemicals

Chloroform, methanol, acetic acid, petroleum ether, diethyl ether, benzene. All are product of Evans Medical PLC, Nigeria.

2.2 Plant Collection and Identification

The stems of *A. compressus*, *A. leiocapus,* and *S. fistula* were obtained from Ilara-Mokin, Akure Ondo State and were identified at the Department of crop science of Federal University of Technology, Akure, Nigeria.The stems of the three plants were shade dried for three weeks at room temperature after which they were pulverized with the aid of a blender, until a fine powdered samples obtained.

2.3 Extraction of Lipid

The lipids in plant were extracted by the modified procedure of Bligh and Dyer as reported by Kates (1970). 5 g of powdered samples were shaken with 18 ml methanol – chloroform (2:1, v/v), for 2minutes. The mixture was filtered and the filtered residue was shaken with 18 ml of methanol-chloroform (2:1, v/v) and 2 ml of H_20. Followed by another round of filtration and the filtered residue was washed with 3 ml of methanol-chloroform (2:1; v/v), to the combined filtrates in a separatory funnel, 5 ml of chloroform and 6 ml of water was added and the phases were allowed to separate. The chloroform layer was withdrawn, diluted with benzene and concentrated in vacuo. The residual lipids were immediately dissolved in 0.1 ml chloroform –methanol (1:1).

2.4 Sample Application on TLC

Sample and standard were applied as discrete spots 0.5 cm from the bottom of the activated plate by means of micro syringe and the loaded plates were air dried

2.5 Sample Develpoment

Solvent 8 – 20 ml was added to the tank and allowed to equilibrate and chamber air for 30 minutes before use. The developing tanks were all glass, with ground glass seal. The tank dimensions were approximately 10 x 30 x 27 cm. The loaded chromatoplates were placed in a developing tank containing the solvents until the solvent had ascended to the top edge of the plate. The solvent moves by capillary action taking the various components with it at different rates, according to the extent to which they are held by the adsorbent. The chromatograms were removed and air-dried in an air current.The solvent system for the neutral lipids consist chloroform, methanol, acetic acid, water in ratio 85:15:10:4 and petroleum ether, diethyl ether acetic acid in ratio 80:20:1 the lipid standards were soya oil and stearic acid (Harbone, 1984).

2.6 Detection of spot using iodine Vapour

The chromatograms were exposed to iodine vapour to make the separated bands visible. The neutral lipids were visualized with iodine vapour brown spots.

2.7 Statistical Analysis

All analysis was run in triplicates. The mean value and standard deviation were calculated using the Microsoft Excel Software (Microsoft Corporation, Redmond, WA).

3. Results and Discussion

Thin layer chromatography is used to identify compounds present in a given mixture and also determines the purity of a substance (Lagarde, 2003). The TLC analysis of the neutral lipids showed the presence of diacylglycerol and frees fatty acids and was further confirmed by applying specific colour reagent. The TLC results confirmed the presence of stearic acid in soy oil; this is in accordance with the findings of Holcapek (2003), who identified stearic acid in orange and guava mistletoes using stearic acid and soy oil as standard, the choice of soy oil is because it contains stearic acid. *Anogeiessus leiocarpus* extract have been found to have separated into more components than *Senna fistula* and *Axonopus compressus* extracts thereby, suggesting that

Anogeiessus leiocarpus are likely to be richer in lipids more than *Senna fistula* and *Axonopus compressus*.

The GC-MS is a confirmation test, to separate chemical substances based on their volatility and the relative amount of such component can also be determined (Baskin, 2005). The fatty acid profiles of the extracted lipids were analyzed by GC-MS as summarized in Table 7, 8 and 9, the results revealed that the fatty acids composition of the three plant extracts are mostly saturated fatty acids. Palmitic acid (C_{16}:0) and stearic (C_{18}:0) acid are the predominant fatty acids in the three plant extracts. This result is consistent with the previous studies showing that the common fatty acids of plant tissues are carbon 16 and carbon 18 (David, 2006). The presence of linoleic acid (C_{18}:2) in *Axonopus compressus* and *Anogeiessus leiocarpus* has made the plants very significant for consumption since the body cannot make it and must be supplied through diet. Linoleic acid is a necessary precursor in mammals for the biosynthesis of arachidonic acid which is not found in plants (Stumpf and Conn, 1980). The presence of lauric acid in the three plant extracts will contributes to good shelf life of the plants (Person, 1994). The total saturated fatty acid level was 73.01% in *Axonopus compressus* 83.16% in *senna fistula* and 82.81% in *Anogeiessus leiocarpus*. The high percentage of saturated fatty acids showed that the lipid is a volatile lipid which supports its potential use in the treatment of haemohoids (Bloor, 1920). The presence of linoleic, oleic and linolenic acids in the plant extracts has been reported to calm inflammation within body (Bloor, 1920)

4. Conclusion

The study has been able to provide scientific support for the medicinal potentials of the stem- bark of *S. fistula, A. leiocarpus* and *A. compressus* which has been demonstrated to contain some levels of essential fatty acids. Therefore, these plants can be harnessed in preventive health care.

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Table 1: TLC separartion of neutral lipids from *Axonopus compressus, Anogeiessus leiocarpus and Senna fistula*

Sample	No of spots	Components	R_f	Inference
Axonopus compressus	3	1	0.15	Diacylglycerol
		2	0.23	
		3	0.32	
Anogeiessus leiocarpus	5	1	0.15	Diacylglycerol
		2	0.23	
		3	0.26	
		4	0.32	
		5	0.50	Free fatty acid
Senna fistula	3	1	0.15	Diacylglycerol
		2	0.23	
		3	0.32	
Standards				
Soy oil	4	1	0.15	Diacylglycerol
		2	0.23	
		3	0.32	
		4	0.50	Free fatty acid
Stearic acid	4	1	0.15	Diacylglycerol
		2	0.23	
		3	0.32	
		4	0.45	Free fatty acid

Solvent: Chloroform-methanol-acetic acid-water (85:15:10:4); Loading volume: 5ml
Staining reagent: Iodine vapor

Table 2: TLC separation of neutral lipids *Senna fistula, Axonopus* compressus and *Anogeiessus leiocarpus*

Sample	No of spots	Components	Rf	Inference
Axonopus compressus	3	1	0.17	Diacylglycerol
		2	0.27	
		3	0.31	
Senna fistula	3	1	0.17	Diacylglycerol
		2	0.27	
		3	0.46	Free fatty acid
Anogeiessus leiocarpus	5	1	0.17	Diacylglycerol
		2	0.25	
		3	0.27	
		4	0.31	
		5	0.46	Free fatty acid
Standards				
Soy oil	4	1	0.17	Diacylglycerol
		2	0.27	
		3	0.31	
		4	0.46	Free fatty acid
Stearic acid	3	1	0.17	Diacylglycerol
		2	0.27	
		3	0.31	

Solvent: Petroleum ether-diethyl ether-acetic acid (85:15:10:4); Loading volume: 5ml
Staining reagents: Iodine vapor

Table 3: Distribution of fatty acids in *A. compressus* extract as identified by GC-MS

Common Name	Systematic Name	No of Carbon	Percentage Composition (%)
Myristic acid	Tetradecanoic acid	14	2.90
Palmitic acid	Hexadecanoic acid	16	26.40
Linoleic acid	9, 12-Octadecadienoic acid	18	16.31
Stearic acid	Octadecanoic acid	18	38.21
Oleic acid	9-Octadecenoic acid	18	2.68
Lauric acid	Dodecanoic acid	12	5.50
Pamitoleic acid	9-Hexadecenoic acid	16	8.00

Table 4: Distribution of fatty acids in *S. fistula* extract as identified by GC- MS

Common Name	Systematic Name	No of Carbon	Percentage Composition (%)
Palmitic acid	Hexadecanoic acid	16	46.63
Linolenic acid	9, 12, 15-Octadecatrienoic acid	18	5.35
Myristic acid	Tetradecanoic acid	14	2.58
Stearic acid	Octdecanoic acid	18	23.25
Oleic acid	9-Octadecenoic acid	18	16.84
Lauric acid	Docanoic acid	12	5.35

Table 5: Distribution of fatty acids in *A. leiocarpus* extract as identified by GC-MS

Common Name	Systematic Name	No of Carbon	Percentage Composition (%)
Linoleic acid	9, 12-Octadecadienoic acid	18	14.25
Palmitic acid	Hexadecanoic acid	16	15.20
Lauric acid	Dodecanoic acid	12	6.92
Capric acid	Decanoic acid	10	3.81
Stearic acid	Octdecanoic acid	18	1 6.84
Oleic acid	9-Octadecenoic acid	18	6.80
Behenic acid	Docosanoic acid	22	10.78
Linolenic acid	9, 12, 15-Octadecatrienoic acid	18	3.79
Myristic acid	Tetradecanoic acid	14	7.78

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