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In-vitro Cholinesterase Inhibition Potential and Fatty Acid Profiling of Lipids Obtained via Direct Trans-esterification from Sarcocephalus latifolius (sm.) Fruits and Leaves

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All authrors declares no conflict of interest

ORIGINAL STUDY

In-vitro Cholinesterase Inhibition Potential and Fatty Acid Profiling of Lipids Obtained Via Direct Trans-esterification from *Sarcocephalus latifolius* (sm.) Fruits and Leaves

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Abstract

***Sarcocephalus latifolius* (sm.) of the family, *Rubiaceae*, is an important plant used in folkloric medicine for the management of various disease conditions. An innovative green route was adopted for the direct characterization of the underutilized tropical plant which was profiled for the fatty acid composition and examined for the *in vitro* anti-cholinesterase potential. The fatty acid methyl esters obtained via direct methylation was characterized using Gas Chromatography-Mass Spectrometry, GC-FID/GC-MS. With donepezil hydrochloride as a reference, the cholinesterase enzyme inhibition capacity of the FAMEs was further assessed through standard assay. Four fatty acids were identified from the total ion chromatogram of the two samples, with 10-methylheptadecanoic acid common to both leaf and fruit. Palmitic acid (41.55 %, 26.89 %) and 10-methylheptadecanoic acid (40.32 %, 2.27 %) were predominant in the leave and fruit with linoleic acid (18.13 %, 20.83 %) also appearing significantly in the leaf and fruit respectively. 8,11,14-heptadecatrienoic acid (29.55 %, RT 16.89) was only detected in the fruit. The presence of linoleic acid, an important polyunsaturated omega-6 fatty acid is presumed to contribute significantly to the anticholinesterase activity of the plant. Although 10-methylheptadecanoic acid was also present in the fruit, it was the least available in it as 8, 11, 14-heptadecatrienoic acid (29.55 %), hexadecanoic acid (26.89 %) and octadeca-9,12-dienoic acid (20.83 %) were more abundant. The leave which had higher extraction yield indicated a reduced activity with IC_{50} of 231.3 ± 1.66 μg/mL compared to the fruit (29.14 ± 12.89) and the standard, donepezil hydrochloride (127.70 ± 5.77 μg/mL). The rapid determination of *S. latifolius* fatty acid profile of the leave and fruit was made possible by the direct trans-esterification method. This green approach is feasible, time saving, consumes less solvents, and lowers the risk of contamination that comes with traditional multistage procedures. Following the exploration of the chemical makeup and the observed antioxidant potential of *S. latifolius* obtained in this study, the folkloric applications is being established. The data obtained in this study revealed that *S. latifolius* contains fatty acids with significant antioxidant potential that warrant further exploration.**

Keywords: Green chemistry, Direct methylation, Acetycholinesterase inhibitors, Lipid, Tropical plant

1. Introduction

The fruits, leaves and seed of plant plays vital roles in human diet and nutrition [1–3]. The application of underutilized tropical plants materials for domestic and industrial applications cannot be overemphasized [4–9]. Fatty acid, FAs often obtained in underutilized seeds and leaves have been reported to possess antioxidant activities and anti-inflammatory activities [10] among others.

Following the discovery of some medicinal plants with good capacity to act as acetycholinesterase inhibitors, the exploration of natural products with such potential is warranted. A

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cholinesterase or choline esterase, refers to an enzyme that catalyses the hydrolysis of an ester that lyses choline-based complex which serve as neurotransmitters [11]. Conventionally, a deficiency in levels of the neurotransmitter acetylcholine is known to induce neurodegenerative diseases and the inhibition of acetylcholinesterase (AChE) is recognized as a major treatment option [12]. Traditionally, plants have been shown to be good options in the search for AChE inhibitors. Recently, several plants have been identified as containing AChEI activity.

S. latifolius of the family, *Rubiaceae*, is a shrub used in folkloric medicine for the management of various diseases which include Jaundice, malaria, high blood pressure, dysentery, aches, snake bites and inflammation [13,14]. The Rubiaceae is a large family comprising over 600 genera and multiple species. Traditionally, the decoction from the plant is used to bath babes to improve resistance to infections. As a relatively obscure plant that thrives majorly in the tropics, there is dearth of information particularly on the chemical composition and their associated bioactivity. The decoction from the root is used as coccidiostat in local poultry farming [15,16]. The plant is also reported for its antioxidant acetylcholinesterase inhibitory activities [14]. Although the biological activity of extracts from *the* plant has been reported, studies that characterizes the bioactive component are rare.

This study explore the profiling of the lipid content of the leave and fruit of the plant using conventional and one-step method. The *in vitro* antioxidant potential of the extract were also evaluated using standard anti-cholinesterase assays. The efficacy of one-step and multistep extraction methods were compared with reference to the yield, chemical composition and antioxidant potential.

2. Materials and methods

2.1. Collection of plant materials

The fruits and leaves of *S. latifolius* were collected within Ilorin metropolis, Nigeria. The plant materials were identified and documented at the Herbarium of the Department of Plant Biology, University of Ilorin, Nigeria where the voucher specimen number, UILH/001/506 was assigned. The materials were cut into small bits and dried at room temperature. For the soxhlet extraction, the dried leaves and dried fruits were also pulverized but used without pulverization for the one-step extraction procedure.

2.2. Solvents and reagents

Analytical grade chemicals which include sulphuric acid, sodium hydroxide (Sigma−Aldrich) and all bioassay reagents used in the study and where applicable, solvents which include methanol and n-hexane were re-distilled before use.

2.3. Extraction of seed oils

The pulverised *S. latifolius* fruits and leaves materials, 100 and 69 g respectively, were separately subjected to soxhlet extraction using n-hexane as the extracting solvent at 60 °C for 2 h. The extract was concentrated via rotary evaporator to obtain the concentrate. The extract from the fruit and leave yielded 0.75 and 5.42 g respectively.

2.4. Innovative one-step extraction and transesterification of lipid from fresh plant

For the one-step, the reaction was performed in one-necked glass reactor (round-bottom flask) equipped with a reflux condenser. A recipe containing 76 mL methanol, 35 mL n-hexane, 11 mL ethyl acetate and 2 mL sulphuric acid was added to the unpulverized sample of *S. latifolius* fruit and leave (60 g and 30 g separately) in the flask respectively. The mixture was refluxed for 30 min in a one neck-flask. The resulting mixture was allowed to cool and then filtered. The supernatant was separated in a separating funnel and the organic layer washed with distilled water to neutrality. The organic layer was concentrated to obtain the transmethylated product: fatty acid methyl esters (FAMEs) and the yield noted. The method which afforded the extraction, hydrolysis and methylation of the lipid in a single procedure is a slight modification of the method previously described [17,18].

2.5. Transesterification of the soxhlet extracted oils

The oil obtained was trans-esterified with 0.1M NaOH following refluxing for 30 min. The fatty acid methyl esters (FAMEs) obtained was extracted with hexane, washed to neutral with water, concentrated and dried over anhydrous sodium carbonate. The extract was thereafter subjected to GC-MS analysis and the yield noted.

2.6. Gas chromatography-mass spectroscopy (GC-MS) analysis

The FAMES obtained from the direct and conventional method were subjected to Gas

Chromatography-Mass Spectrometry (GC-MS) analysis in order to identify the compounds present in them. Approximately, 1.0 g sample was diluted in 8.0 mL hexane and further to a microgram unit before injection into the Shimadzu GC-2010 gas chromatograph. The parameters for the analysis were as indicated: The detector, FID at 220 °C, N_2 flowing at 1.0 mL/min on a SPB-5 capillary column (30 m × 0.53 mm ID; 0.32 mm) with split ratio 1:30 and injector temperature 250 °C, and column temperature maintained at 40 °C for 5 min before increasing to 280 °C (5 °C/min) with a delay at that temperature for an additional 5 min. For the GC-MS, a Jeol JMS-HX 110 mass spectrometer with a source at 270 °C and 70 eV coupled with a Hewlett–Packard 6890 gas chromatograph was used. The injector was configured to split at 1:30 and temperature at 270 °C. Compound identification was established by comparing the MS fragmentation with authentic samples and data obtained on the NIST mass spectral software 2008 database.

2.7. Anti-cholinesterase activity

The anti-cholinesterase inhibitory assay analysis was conducted following standard protocol [12]. The assay mixture consisted of 200 µL of Tris-HCL 50 mM pH 8.0, 0.1 % BSA buffer, 100 µL of n-hexane extract solution (final concentration: 100 gmL^{-1}) will be dissolved in buffer-MeOH (10 %) and 100 µL of anti-cholinesterase (0.22 U mL^{-1}). The mixture was incubated at room temperature for 2 min before the addition of 500 µL of DTNB (5,5 dithiobis [2-nitrobenzoic acid] (3 mM) and 100 µL of substrate acetyl thiocholine iodide (ATCI) (15 mM). The developing yellow colour was measured at 405 nm after 4 min. Donepezil Hydrochloride was used as positive control at a final concentration of 0.2 μgmL^{-1} in the assay mixture. Anti-cholinesterase inhibitory activity express as per cent inhibition of anti-cholinesterase calculated as (1-B/A) X 100, where A is the change in absorbance of the assay without the plant extract (abs. with enzyme − abs. without enzyme) and B is the change in absorbance of the assay with the plant extract (abs. with enzyme − abs. without enzyme).

2.8. Data analysis

The bioassay data was measured in triplicate and reported as average with standard error of mean (SEM). The concentration of the sample exhibiting 50 % reduction in activity (i.e. IC_{50} values) was determined using Prism 5 (GraphPad; CA, USA) using a non-linear regression analysis for fitting.

3. Results and discussion

3.1. GC-MS analysis results

FAMEs were obtained from the fruit and leaves of *S. latifolius* via the conventional and multi-step techniques (Fig. 1). The yield obtained is as reported (Table 1). 10-methylheptadecanoic acid common to both. Palmitic acid and 10-methylheptadecanoic acid were predominant in the leave at a relative abundance of 41.55 and 40.32 % respectively with linoleic acid (18.13 %) also appearing significant. Other fatty acids were below the detection limit. Although 10-methylheptadecanoic acid was also present in the fruit, it was the least available in it as 8, 11, 14-heptadecatrienoic acid (29.55 %), hexadecanoic acid (26.89 %) and octadeca-9,12-dienoic acid (20.83 %) were more abundant.

The results obtained from GC-MS of the one-step extraction method is as indicated (Table 2). The result showed the presence of four [4] fatty acids in all. The FAMEs obtained via the direct and multi-steps extraction methods revealed the presence of fatty acids which include palmitic acid (41.55 %, 26.89 % RT 15.66), linoleic acid (18.13 %, 20.83 %, RT 16.84) and 10-methylheptadecanoic acid (40.32 % and 2.27 %, RT 17.04) in the leaf and fruit respectively while 8,11,14-heptadecatrienoic acid (29.55 %, RT 16.89) was only detected in the fruit. While the other fatty acids were below the detection limit, the amount of fatty acids in leaf and fruits are often less

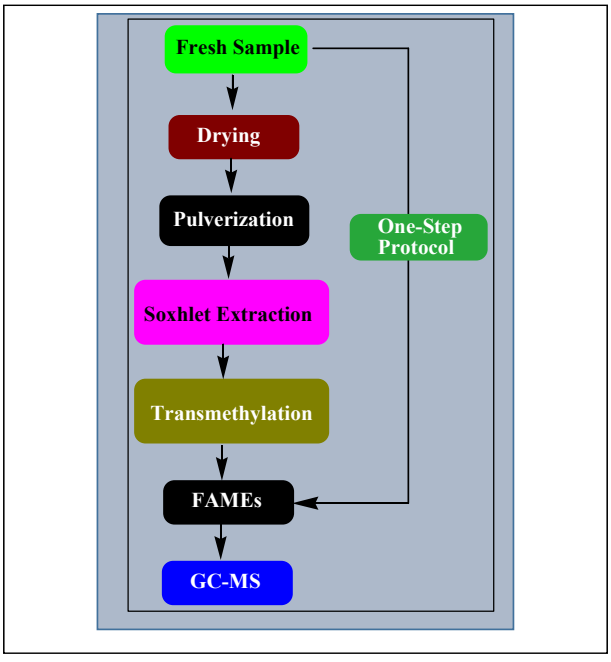

Fig. 1. Comparison of conventional and one-step protocols.

Table 1. FAMEs extraction yield.

Yield of One-step Extraction - Leaves	Yield of Multi-steps Extraction - Leaves	Yield of One-step Extraction - Fruits	Yield of Multi-steps Extraction - Fruits
5.42 %	1.09 %	0.11 %	1.12 %

Table 2. Fatty acid composition of S. latifolius *leaves and fruits extract.*

S/N	Retention Time	Name of Compound	%Composition (Leave)	%Composition (Fruit)
1.	15.66	Palmitic acid	41.55	26.89
2.	16.84	Linoleic acid	18.13	20.83
3.	16.89	8, 11, 14-heptadecatrienoic acid	–	29.55
4.	17.04	10-methylheptadecanoic acid	40.32	2.27

Table 3. Inhibition of acetyl-cholinesterase activity by S. latifolius *leaf and fruit FAMEs.*

Conc (μg/ml)	%Inhibition (Leave)	%Inhibition (Fruit)	%Inhibition (Donepezil)
10	30.56 ± 3.57	24.90 ± 5.82	16.66 ± 2.94
20	34.49 ± 15.56	29.16 ± 19.66	24.69 ± 6.51
50	35.78 ± 14.94	33.65 ± 7.60	35.97 ± 1.40
100	33.66 ± 2.47	37.18 ± 4.56	40.86 ± 2.77
150	40.71 ± 6.81	40.18 ± 8.1	49.10 ± 7.19
IC_{50} values ± SEM (μg/mL)	231.3 ± 1.66	29.14 ± 12.89	127.70 ± 5.77

compared to seed which naturally houses more lipid content of plants.

3.2. In vitro *anti-cholinesterase activity*

The leaf extract did not exhibit a dose-dependent cholinesterase inhibition potential particularly when there was a decline at 100 μg/mL concentration (see Table 3). The fruit extract however showed dose-response inhibition that comparable to the standard, donepezil hydrochloride. While the activity of the fruit extract ranges 24.90−40.18 % at 10 and 150 μg/mL respectively, it ranges at 16.66 and 49.10 % at the same concentration in the standard. The leaves extract which had higher extraction yield indicated a reduced activity with IC_{50} of 231.3 ± 1.66 μg/mL compared to the fruit (29.14 ± 12.89) and the standard, donepezil hydrochloride (127.70 ± 5.77 μg/mL).

The combination of extraction and trans-esterification into a single phase, direct trans-methylation (DTM) allows for a quicker, easier analysis with a lower solvent consumption. Better recovery of the sample's total fatty acid methyl ester is achieved by the approach, which has no bias or selectivity toward any particular lipid class. Organic solvent extraction is reported to be significantly more effective if the plant samples are first crumpled and dried, with the DTM eliminating the need for full drying. According to reports, the DTM process complies with the requirements of the Nutritional Labeling and Education Act (NLEA) in the United States [19]. Hexane and benzene are the solvents in this study's recipe, which also includes sulfuric acid for hydrolysis, aluminum chloride as a catalyst, and methanol for methylation. As a result, the FAMEs were produced in a single process with a yield of 5.42 % and 0.11 for the leaves and fruit of the fresh material respectively. Although, due to the low yield observed in the fruit, the direct extraction protocol may not be suitable for the characterization of the wet fruit.

The different fatty acids in the plant may now be quickly identified in a single process following the direct trans-esterification of the lipid and subjection to GC-MS analysis. The extraction process in one step appears to be effective, inexpensive, and time-saving for analyzing the lipid particularly the leave. Linoleic acid, an important polyunsaturated fatty acid found in both leaf and fruit was significant. The underutilized tropical leave and fruit exhibiting significant anti-inflammatory activity, suggests that the plant could be a natural renewable source of antioxidants. However, more research is necessary to confirm the biological viability and safety profile.

3.3. Conclusion

The fatty acid constituents of the fruit of *S. latifolius* have been investigated in this study. The extract in the leave and fruit of the plant was extracted via one-step extraction and conventional multi-steps extraction methods and analyzed using GC-MS. The rapid extraction and profiling is an innovative green approach that saves time, prevent use of large quantity of toxic chemicals that have negative implication on the environment.

Data availability

No other dataset to declare.

Ethics information

All protocols adopted follows standard ethical procedure.

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Conflict of interest

Authors declare no conflict of interest.

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References

[1] Adeyemi KD, Sola-Ojo FE, Ahmed El-Imam AM, Atolani O, Ali OI, Adegboyega AO, et al. Dietary oil type and late feed restriction elicit synergistic effects on growth, caeca bacteria, carcass, fat accretion, and muscle lipids in female broilers. Eur J Lipid Sci Technol 2023;125(6):2200216. https://doi.org/10.1002/ejlt.202200216.

[2] Atolani O, Olorundare OE, Anoka AN, Osin AO, Biliaminu SA. Antioxidant, proteinase inhibitory and membrane stabilization potentials of *Moringa oleifera* seed oil. Fabad J Pharm Sci 2018;43(2):1–13.

[3] Atolani O, Areh ET, Oguntoye OS, Zubair MF, Fabiyi OA, Oyegoke RA, et al. Chemical characterization, antioxidant, cytotoxicity, anti-toxoplasma *gondii* and antimicrobial potentials of the *Citrus sinensis* seed oil for sustainable cosmeceutical production. Heliyon 2020;6(2):e03399.

[4] Zubair MF, Atolani O, Ibrahim SO, Oguntoye OS, Oyegoke RA, Olatunji GA. Fatty acids composition, antimicrobial potential and cosmeceutical utilization of prosopis africana seed oil. 2019 J Mex Chem Soc 2018;62(3).

[5] Zubair MF, Ibrahim SO, Stephen K, Hamid AA, Ibukun O, Atolani O. Synthesis and chemical characterization of alkyd resins using maleic and phthalic anhydrides and seed oil of *Luffa aegyptiaca*. J Turkish Chem Soci 2023. Section A.

[6] Zubair MF, Ibrahim SO, Atolani O, Hamid AA, Ibukun OJ, Abdulrahim HA. Chemical characterization, preparation of biosurfactant and biochemical evaluation of seed oil of *Luffa aegyptiaca*. Chemist 2022;93(1).

[7] Atolani O, Olatunji GA, Fabiyi OA, Adeniji JA, Ogbole OO. Phytochemicals from *Kigelia pinnata* leaves show antioxidant and anti-cancer potentials on human cancer cell line. J Med Food 2013;16(10):878–85.

[8] Oguntoye SO, Ezennaya OL, Yusuff OK, Atolani O. Eco-friendly formulation, characterizations, bioactivity studies and *In silico* evaluation of cosmetic prepared from the seed oils of *Carica papaya, Dacryodes edulis* and *Raphia hookeri*. Chemists, J American Instit Chem 2023;94(2).

[9] Atolani O, Olabiyi ET, Issa AA, Azeez HT, Onoja EG, Ibrahim SO, et al. Green synthesis and characterization of natural antiseptic soaps from the oils of underutilized tropical seed. Sustain Chem Pharm 2016;4:32–9.

[10] Atolani O, Oguntoye H, Areh ET, Adeyemi OS, Kambizi L. Chemical composition, anti-toxoplasma, cytotoxicity, antioxidant, and anti-inflammatory potential of *Cola gigantea* seed oil. Pharmaceut Biol 2019;57(1):154–60.

[11] Colovic MB, Krstic DZ, Lazarevic-Pasti M, Bondzic AM, Vasic VM. Acetycholinesterase inhibitors: pharmacology and toxicology. Current Neuropharm 2013;11(3):315–35. Bentham Science publishers Ltd.

[12] Şenol FS, Orhan İ, Yilmaz G, Çiçek M, Şener B. Acetylcholinesterase, butyrylcholinesterase, and tyrosinase inhibition studies and antioxidant activities of 33 Scutellaria L. taxa from Turkey. Food Chem Toxicol 2010;48:781–8.

[13] Ngo Bum E, Taiwe GS, Motto FC, Ngoupaye GT, Nkantchoua GC, Pelanken MM, et al. Anticonvulsant, nxiolytic and sedative properties of the roots of Nauclea latifolia smith in mice. Epilepsy Behav 2009;15(4):434–40.

[14] Osama A, Awadelkarim S, Ali A. Antioxidant activity, acetylcholinesterase inhibitory potential and phytochemical analysis of *Sarcocephalus latifolius* Sm. bark used in traditional medicine in Sudan. BMC Compl Alternative Med 2017;17:270.

[15] Tchodo FG, Dakpogan HB, Adjei-Mensah B, N'nanle O, Karou S, Pitala W, et al. In ovo toxico-pathological effects of medicinal plants used against coccidiosis on chicken embryos development and hatchability. Poultry Sci 2024 14; 103(12):104435. https://doi.org/10.1016/j.psj.2024.104435. Epub ahead of print. PMID: 39515114.

[16] Tchodo FG, Dakpogan HB, Sanvee S, Adjei-Mensah B, Kpomasse CC, Karou S, et al. The anticoccidial in vitro effects and antioxidant properties of several plants traditionally used for coccidiosis in Togo. Vet Sci 2024 Jul 31;11(8):345. https://doi.org/10.3390/vetsci11080345.

[17] Atolani O, Adeniyi O, Kayode OO, Adeosun CB. Direct preparation of fatty acid methyl esters and determination of in vitro antioxidant potential of lipid from fresh sebal causarium seed. J Appl Pharmaceut Sci 2015;5(3):24–8.

[18] Garces R, Mancha M. One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. Anal Biochem 1993;211(1):139–43.

[19] Xiao L. Evaluation of extraction methods for recovery of fatty acids from marine products. In: Master thesis of EMQAL project. University of Bergen: Norway; 2010. p. 4–17.