Science World Journal Vol. 20(No 1) 2025 https://dx.doi.org/10.4314/swj.v20i1.48
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC DETERMINATION OF SOME BIOCHEMICAL COMPONENTS FROM *CROSSOPTERYX FEBRIFUGA* POWERED EXTRACTED USING DIFFERENT MEDIA

*O.N. Efusiauten, M.B. Ardo and A.G. Bobbo

Department of Animal Science and Range Management, Modibbo Adama University, Yola, Adamawa State, Nigeria

*Corresponding Author Email Address: obadiahnoah@yahoo.co.uk

ABSTRACT
This research was undertaken to determine the phytochemical composition of *Crossopteryx febrifuga* stem bark using various extraction methods and Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The stem bark of *C. febrifuga* was collected from Sebore farm, formally identified, shade-dried at room temperature, and ground into fine powder using pestle and mortar. Three extraction methods using ethanol, ethyl acetate, and distilled water were carried out. The extracts were analyzed using Gas Chromatography-Mass Spectrometry. GC-MS results revealed the presence of phytosterols - β-sitosterol and genistein in the ethanolic extract only, while fatty acid esters (precursors of prostaglandins) were present in all three different extracts. In conclusion, the extracts contain phytochemicals with potential applications in veterinary medicine, particularly for reproductive health. Further studies on pharmacological activities of the plant should be investigated in animals.

Keywords: *Crossopteryx febrifuga*, Gas Chromatography-Mass Spectrometry (GC-MS), Ethanol, Ethyl Acetate, Water Extract

INTRODUCTION

Extraction of medicinal plants is a process of separating active plant materials or secondary metabolites such as alkaloids, flavonoids, terpenes, saponins, steroids, and glycosides from inert or inactive materials using appropriate solvent and standard extraction procedure (Abubakar and Hague, 2020). Several methods are used in the extraction of medicinal plants such as maceration, infusion, decoction, percolation, digestion, and Soxhlet extraction. In addition, thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), paper chromatography (PC), and gas chromatography (GC) are used in separation and purification of secondary metabolites.

Gas Chromatography-Mass Spectrometry (GC-MS) is an analytical technique that combines the separation capabilities of gas chromatography (GC) with identification power of mass spectrometry (MS) to identify and quantify unknown substances within a sample (Turner *et al.*, 2020; Pasternak *et al.*, 2022). It is widely used in various fields including forensic science, pharmaceutical research, toxicology, environmental analysis, food safety and chemical analysis due to its high sensitivity and ability to identify complex mixtures (Hernandez *et al.*, 2013; Narciso *et al.*, 2019).

Crossopteryx febrifuga (Afzel. ex G. Don) Benth. (family Rubiaceae) is a small tree widely distributed in the savanna regions of tropical Africa. Traditionally, various parts of the plant have been used in ethnomedicine for treating fever, respiratory disorders, and reproductive health issues in both humans and animals. This study focuses on identifying the phytochemical constituents that may contribute to its reported therapeutic effects, particularly those with potential veterinary applications.

MATERIALS AND METHODS

The experiment was carried out in the Biochemistry Lab of Modibbo Adama University Yola, located within Girei LGA of Adamawa State, Nigeria. The area has a tropical climate with temperatures ranging from 26.9°C to 42°C and annual rainfall of 1500-2000 mm concentrated between April and October. The study area falls within the North Guinea Savannah vegetation zone characterized by high grassland with shrubs and fewer trees (Adebayo *et al.*, 2020).

Plant Collection and Identification

The stem bark of *C. febrifuga* plant was collected from Sebore farm, Mayobelwa Local Government Area of Adamawa State. The plant was formally identified by a botanist from the Department of Forestry and Wildlife of Modibbo Adama University Yola, and a voucher specimen (number CF-2025-007) was deposited in the university herbarium. Three mature plants were sampled, with approximately 500g of stem bark collected from each plant to ensure adequate representation. The stem bark was shade-dried at room temperature (25±2°C) for 14 days during the dry season (January 2025). The sample was ground into fine powder using pestle and mortar. The powdered plant sample was stored in airtight containers at room temperature and used for extraction within one week of processing.

Extraction Methods

Three extraction methods were used: Solvent extraction using ethanol, Solvent extraction using ethyl acetate and Extraction using distilled water as the solvent. For each extraction method, 100g of the powdered plant material was mixed with 500mL of the respective solvent in a conical flask. The mixtures were macerated for 72 hours with occasional shaking. The extracts were filtered using Whatman No. 1 filter paper, and the filtrates were concentrated using a rotary evaporator at 40°C. The concentrated extracts were stored at 4°C until further analysis.

Science World Journal Vol. 20(No 1) 2025 https://dx.doi.org/10.4314/swj.v20i1.48
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Identification of Phytochemical Constituents in *C. febrifuga* Using GC-MS

Phytochemical analysis was conducted by Gas Chromatography-Mass Spectrometry (GC-MS) for the three different extracts. A machine model: GC 7890B, MSD 5977A, Agilent Tech. was used. The GC-MS conditions were as follows: column DB-5MS (30m × 0.25mm × 0.25µm film thickness); carrier gas helium (flow rate 1mL/min); injection volume 1µL; injection temperature 250°C; oven temperature program 60°C (2 min), then increased to 280°C at a rate of 10°C/min with a hold time of 5 min. The MS was operated in electron impact mode at 70eV with an ion source temperature of 230°C.The quantification of all the identified components was investigated using percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time in minutes and mass spectra with those of the National Institute of Standards and Technology (NIST) and Wiley library data of the GC-MS system as described by Hebber and Nalini (2020); Thamer and Thamer (2024).

RESULTS

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Ethanol Extract of *C. febrifuga* (Stem Bark)

The GC-MS analysis revealed the presence of 13 compounds (active principles) with their retention time (RT), peak area percentage, compound name, compound nature, molecular formula and molecular weight (MW). The prevailing compounds in the ethanol extract as shown in Table 1 below are the phytosterols such as β-sitosterol, genistein (an isoflavone), and fatty acid esters which are the precursors of prostaglandins.

Table 1: Chemical Constituents found in Ethanol Extract of *C. febrifuga* (stem bark)

S/ N	Retention Time (Min)	Area (%)	Compound Name	Molecular Formular	Compound Nature	Molecular Weight (gram/mol)
1	1.422	16.01	2(5H)-Furanone, 3-chloro-5-((dimethylamino)methyl)-4,5dimethyl-	C9H14ClNO2	Heterocyclic compound	203
2	2.022	77.33	Ethane, 1-ethoxy-1-methoxy-	C5H12O2	Ether	104
3	3.138	17.3	β-Sitosterol	C29H50O	Steroid	414
4	5.529	1.64	4-Nonene, 3-methyl-, (Z)-	C10H20	Alkene	140
5	8.883	4.91	Hexadecanoic acid, methyl ester	C17H34O2	Fatty acid ester	270
6	12.295	3.77	Cyclohexanecarboxaldehyde, 4-(hydroxymethyl)-	C8H14O2	Cyclic aldehyde	142
7	15.451	2.34	9,15-Octadecadienoic acid, methyl ester, (Z,Z)-	C19H34O2	Fatty acid ester	294
8	17.413	7.9	9-Dodecenoic acid, methyl ester, (E)-	C13H24O2E	Fatty acid ester	212
9	18.313	1.2	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	C16H32O2	Fatty acid ester	256
10	19.727	1.35	Trimethyl[4(1,1,3,3,tetramethyl butyl)phenoxy]silane	C17H30OSi	Silyl ether	278
11	19.8	8.57	Genistein	C15H10O5	Isoflavone	270
12	20.129	2.26	1,2,4-Benzenetricarboxylic acid, 1,2-dimethyl ester	C11H10O6	Aromatic carboxylic acid ester	238
13	25.467	100	3(2H)-Benzofuranone, 6methoxy-2-[(3-methoxyphenyl) methylene]-, (E)-	C17H14O4	Heterocyclic compound	282

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Ethyl Acetate Extract of *C. febrifuga* (Stem Bark)

The GC-MS analysis revealed the presence of 15 compounds (active principles) with retention time (RT), peak area percentage, compound name, compound nature, molecular formula and

Science World Journal Vol. 20(No 1) 2025 https://dx.doi.org/10.4314/swj.v20i1.48
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

molecular weight (MW). The prevailing compound in ethyl acetate extract of *C. febrifuga* (stem bark) as shown in Table 2 is methyl ester, which is also a fatty acid and the precursor of prostaglandin.

Table 2: Chemical Constituents found in Ethyl Acetate Extract of *Crossopteryx febrifuga* (stem bark) using GC-MS Analysis.

S/N	Retention Time (min)	Area (%)	Compound Name	Compound Nature	Molecular Formula	Molecular Weight (g/mol)
1	6.321	1.38	Nonadecane	Saturated hydrocarbon	$C_{19}H_{40}$	268
2	8.503	1.37	1-Nonadecene	Unsaturated hydrocarbon	$C_{19}H_{38}$	266
3	8.576	2.17	10-Heneicosene (c,t)	Unsaturated hydrocarbon	$C_{21}H_{42}$	294
4	10.67	1.84	Tetracosane	Saturated hydrocarbon	$C_{24}H_{50}$	338
5	10.736	1.79	1-Hexadecanol	Long-chain fatty alcohol	$C_{16}H_{34}O$	242
6	11.578	1.19	Phthalic acid, cyclobutyl tridecyl ester	Ester	$C_{25}H_{40}O_4$	402
7	12.047	4.2	Hexadecanoic acid, methyl ester	Ester	$C_{17}H_{34}O_2$	270
8	12.677	1.16	1-Eicosanol	Long-chain fatty alcohol	$C_{20}H_{42}O$	298
9	13.709	1.01	9,15-Octadecadienoic acid, methyl ester, (Z,Z)-	Methyl ester	$C_{19}H_{34}O_2$	294
10	13.768	4.81	9-Dodecenoic acid, methyl ester, (E)-	Methyl ester	$C_{13}H_{24}O_2$	212
11	14.251	2.41	1-Hexyl-1-nitrocyclohexane	Fatty alcohol	$C_{12}H_{23}NO_2$	213
12	21.697	2.03	1-Cyclohexylnonene	Unsaturated hydrocarbon	$C_{15}H_{28}$	208
13	23.564	100	1,15-Pentadecanediol	Diol	$C_{15}H_{32}O_2$	244
14	24.121	5.06	Phthalic acid, di(2-propylpentyl) ester	Ester	$C_{24}H_{38}O_4$	390
15	24.736	1.63	4-Norcarane-2-one, 1,3,5-tri-tert-butyl-3-[1,3,5-tri-tert-butyl-4-oxo-2,5-cyclohexadien-1-yl)methyl]-	Ketones	$C_{38}H_{62}O_2$	550

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Water Extract of *C. febrifuga* (Stem Bark)

The GC-MS analysis revealed the presence of 20 compounds (active principles) with retention time (RT), peak area percentage, compound name, compound nature, molecular formula and molecular weight (MW). The prevailing compounds as shown in Table 3 are methyl tetradecanoate, tridecanoic acid, and hexadecanoic acid which are fatty acid esters, the precursors of prostaglandins (PGF2α).

Table 3: Chemical Constituents found in Aqueous Extract of *C. febrifuga* (Stem Bark)

S/N	Retention time (min)	Area (%)	Compound name	Compound nature	Mol formula	Mol weight (g/mol)
1	1.327	1.04	2-Ethylprop-1-ene (1-3)sultine	Sultine	$C_5H_8O_3S$	132
2	2.037	100	Silane, ethyl-	Organosilicon	C_2H_8Si	60
3	6.313	2.85	N,N-Dimethyl-10-undecen-1-amine	Amine	$C_{13}H_{27}N$	197
4	7.331	1.13	Hydroxylamine, O-decyl-	Hydroxylamine	$C_{10}H_{23}NO$	173
5	7.748	1.2	Nonadecane	Saturated hydrocarbon	$C_{19}H_{40}$	268
6	8.502	2.26	Propanoic acid, 2-(aminooxy)-	Carboxylic acid	$C_3H_7NO_3$	105
7	8.575	3.37	Nonanoic acid	Carboxylic acid	$C_9H_{18}O_2$	158
8	9.234	1.41	Boronic acid, ethyl-	Organoboron	$C_2H_7BO_2$	74
9	9.974	1.41	1-Nonadecene	Unsaturated hydrocarbon	$C_{19}H_{38}$	266
10	10.669	3.11	Cetene	Alkene	$C_{16}H_{32}$	224
11	10.735	2.74	Tetracosane	Saturated hydrocarbon	$C_{24}H_{50}$	338

12	11.57	1.5	Cyclopentanecarboxylic acid	Cyclopentane derivative	$C_8H_{14}O_2$	142
13	12.046	42.52	Methyl tetradecanoate	Ester	$C_{15}H_{30}O_2$	242
14	12.317	1.52	Tridecanoic acid, methyl ester	Ester	$C_{14}H_{28}O_2$	228
15	12.471	2.52	10-Heneicosene (c,t)	Unsaturated hydrocarbon	$C_{21}H_{42}$	294
16	12.683	2.73	Didodecyl phthalate	Phthalate	$C_{32}H_{54}O_4$	502
17	12.734	2.2	Isoxazolidine, 5-ethyl-2,4-dimethyl-, trans-	Cyclic compound	$C_7H_{15}NO$	129
18	13.715	10.33	Hexadecanoic acid, methyl ester	Ester	$C_{17}H_{34}O_2$	270
19	13.767	54.34	3,4-Nonadien-6-yne, 5-ethyl-3-methyl-	Complex unsaturated hydrocarbon	$C_{12}H_{18}$	162
20	13.913	1.03	7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin	Coumarin derivative	$C_{14}H_{14}O_3$	230

Note: The area percentage values represent relative percentages within each extract's chromatogram and are normalized to the most abundant peak in each extract.

DISCUSSION

Gas Chromatography-Mass Spectrometry Analysis of Phytochemical Constituents

GC-MS analysis is a powerful analytical technique for identifying compounds in complex biological matrices. The present study utilized this technique to analyze three different extracts of *C. febrifuga* stem bark, revealing the presence of various bioactive compounds with potential therapeutic applications.

The presence of β-sitosterol in *C. febrifuga* (stem bark) is in agreement with Chouna *et al.* (2015). β-sitosterol is a sterol found in almost all plants. It is one of the main subcomponents of a group of plant sterols known as phytosterols (David, 2018). Phytosterols are structurally similar to cholesterol, occurring in plants and vary in absence or presence of a double bond in carbon side chain. These phytosterols do not produce undesirable side effects and are generally recognized as safe (GRAS). More than 200 sterols and allied compounds have been identified (Gupta, 2020). Cholesterol is mainly synthesized in large quantities in the liver of animals and only in small quantities by plants. In contrast, sitosterol is only synthesized by plants (Kraus, 2020).

Some clinical and preclinical studies suggest that β-sitosterol provides many health benefits. It lowers the level of bad cholesterol (LDL) and reduces the risk of coronary artery disease, heart attack and atherosclerosis, preventing many types of cancers along with supporting body's natural recovery process (Gupta, 2020). Other health benefits include reduction of swelling in the prostate and improvement of urinary symptoms, reduction of inflammation by inhibiting hormonal influences that trigger inflammation, and antioxidant properties that may have protective effects on digestive diseases. Sitosterol may help with insulin resistance and metabolic disorders (Ribbing *et al.*, 2010).

The presence of genistein (an isoflavone) in *C. febrifuga* (stem bark) is of importance in reproduction and health. Genistein is a phytoestrogen that, due to its structural similarity with estrogen, can both mimic and antagonize estrogen effects depending on the concentrations (Mas-Borgues and Vina, 2022). Early analysis proved that at high concentrations, genistein inhibits breast cancer cell proliferation, thereby suggesting an anticancer activity. Since then, many discoveries have identified the genistein mechanism of action including cell cycle arrest, apoptosis induction as well as angiogenesis and metastatic inhibition (Chen *et al.*, 2015).

Genistein has many potential health benefits, including reduction in the risk of cardiovascular disease, alleviating post-menopausal symptoms, protecting against osteoporosis, inhibiting breast cancer cell proliferation, promoting autophagy. It also acts as antioxidant and anti-inflammatory and has beneficial effects in age-associated diseases, such as Alzheimer's disease (Chen *et al.*, 2015; Pawlicki *et al.*, 2022; Gao *et al.*, 2022).

The presence of fatty acids in *C. febrifuga* generally plays an important role in modulation of reproduction potential in livestock. These compounds help in follicular development, oocyte development and maturation, and also provide oxidative energy supply in oocyte development and maturation. The health and reproductive performance of livestock are directly affected by maternal nutritional and physiological condition throughout gestation and lactation (Butter, 2000). Nutrient shortages and excesses can cause reproductive disturbance and influence reproductive performance (Bisinotto *et al.*, 2012; Prunier and Quesnel, 2000).

The role of specific fatty acids involved in the biosynthesis of prostaglandins is important. Prostaglandins belonging to the eicosanoids group are 20-carbon unsaturated hydroxyl fatty acids with cyclopentane ring, the main precursor of prostaglandins (McCracken, 2005). The significance of fatty acids in modulating the reproductive potential of livestock has received greater recognition in recent years. Functional fatty acids and their metabolites improve follicular development, oocyte maturation and embryo development, as well as endometrial receptivity and placental vascular development, through enhancing energy supply and precursors for the synthesis of reproductive hormones, such as steroid hormones and prostaglandins (Zeng *et al.*, 2023).

Conclusion

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the three different extracts (ethyl acetate, ethanolic and aqueous) of *C. febrifuga* revealed significant phytochemical constituents. The ethanolic extract contained important phytosterols such as β-sitosterol and the isoflavone genistein, which have been reported to possess estrogenic properties. All three extracts contained fatty acid esters that are known precursors of prostaglandins (PGF2α). These bioactive compounds have potential applications in veterinary medicine, particularly in reproductive health management, as they may influence hormonal activity. The

presence of these compounds provides scientific evidence supporting the traditional veterinary uses of *C. febrifuga* stem bark. Therefore, the stem bark extracts of *C. febrifuga* contain biologically active compounds with potential applications in veterinary medicine and animal reproductive health.

Recommendations

Gas Chromatography-Mass Spectrometry (GC-MS) should be carried out to determine the phytoconstituents in plants that may be useful to farm animals before their application in ethnoveterinary medicine. Based on the presence of fatty acids (precursors of PGF2α) which can potentially influence cervical dilation and uterine contraction, *C. febrifuga* (stem bark extract) may have applications in reproductive health management of farm animals. However, controlled clinical studies are necessary before specific veterinary applications can be recommended. Further studies on the pharmacological activities, safety profile, and effective dosages of *C. febrifuga* extracts should be investigated in animals to validate its traditional uses and establish its therapeutic potential.

REFERENCES

Abubakar, A. R. and Hague, M. (2020). Preparation of medicinal plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *Journal of Pharmacy and Bio-allied Sciences*, 12(1), 1-10.

Adebayo, A. A. (2010). Climate: Resource and resistance to Agriculture. Eight Inaugural Lecture Federal University of Technology, Yola. Adamawa State, Nigeria.

Adebayo, A. A., Tukur, A. L., and Zemba, A.A. (2020) Climate I, sunshine, temperature, evaporation and relative humidity, In: Adebayo A. A. and Tukur A. L (editors) Adamawa State in maps. Second edition (2020). Yola, Nigeria: Paraclete Publishers.

Alexander, B. C. (2015). Comparative Analysis of thunder storm and rainfall occurrence over Nigeria. International Organization of scientific research (OISR). *Journal of Environmental Science, Toxicology and Food Technology*, 9(3), 33-44.

Bisinotto, R.S., Greco, L.F., Ribeiro, E.S., Martinez, N., Lima, F.S., Stapies, C.R., et al. (2012). Influence of nutrition and metabolism on fertility of dairy cows. *Journal of Animal Reproduction*, 9(3), 260-272.

Butter, W. (2000). Nutritional interactions with reproductive performance in dairy cattle. *Journal of Animal Reproductive Science*, 60, 449-457.

Chen, J., Duan, Y., Zhang, X., Ye, Y. and Ge, B. (2015). Genistein induces apoptosis by the inactivation of the IGF-1R/P-Akt Signaling pathway in MCF-7 human breast cancer cells. *Food Function*, 6, 995-1000.

Chouna, J.R., Tamokou, J., Nkeng-Efouet-Alango, P., Lenta, B.N. and Sewald N. (2015). Antimicrobial triterpenes from the stem bark of *Crossopteryx febrifuga*. *Journal for Nature Research*, 70(7-8), 169-173.

David R.M.D. (2018). Benign Prostatic Hyperplasia. *Integrative Medicine* (Fourth Edition).

Gao, H., Lei, X., Ye, T., Hua, R., Wang, G., Song, H., Zhau, P., Wang, Y. and Cai, B. (2022). Genistein attenuates memory impairment in Alzheimer's disease via ESR-Mediated apoptotic pathway *in vivo* and *in vitro*. *Journal of Nutritional Biochemistry*, 109, 108569.

Gupta, E. (2020). β-Sitosterol: predominant phytosterol of Therapeutic Potential. *Innovations in Food Technology*, 465-477.

Hebber, D.R. and Nalini, M.S. (2020). Characterization of anti-oxidative compounds from the stem bark and flower extracts of *Scheffera species* from Western Ghats. *Der Pharmaceutical Letters*, 12, 51-60.

Hernandez, F., Cervera, M.I., Portoles, T., Beltran, J. and Pitarch, E. (2013). The role of GC-MS/MS with triple quadrupole in pesticide residue analysis in food and the environment. *Analytical Methods*, 5(21), 5875-5894.

Kraus, J. (2020). Cholesterol and Sitosterol. Two members of the steroid family that differ in structure by a single Ethyl group yet maintain the right balance between Plant and Animal Kingdoms. *Russian Journal of Bioorganic Chemistry*, 46, 869-872.

Mas-Bargues, C. and Vina, J. (2022). The Multi modal action of Genistein in Alzheimer's and other age-related diseases. *Free Radical Biology and Medicine*, 188, 134-143.

McCracken, J. A. (2005). Prostaglandins and Leukotrienes. In Endocrinology: Basic and Clinical Principles; Humana Press; Totowa New Jersey, USA, 93-111.

Narciso, J., Luz, S. and Bettencourt da Silva R. (2019). Assessment of the quality of doping substances identification in urine by GC/MS/MS. *Journal of Analytical Chemistry*, 91(10), 6638-6644.

Pasternak, Z., Avissar, Y.Y., Ehila, F. and Grafit, A. (2022). Automatic detection and classification of Liquids from GC-MS data of Casework Samples in forensic fire-debris analysis. *Journal of Forensic Chemistry*, 29, 100419.

Pawlicki, M.A., Marciniec, S.P. and Filip, A. (2022). The effects of Genistein at Different Concentrations on MCF-7 Breast cancer cells and BJ Dermal Fibroblasts. *International Journal of Molecular Science*, 23(20), 12360.

Prunier, A. and Quesnel, H. (2000). Influence of the nutritional status on ovarian development in female pigs. *Journal of Animal Reproductive Science*, 60, 185-197.

Ribbing, J., Hamren, B., Svenson, M.K. and Karisson M.O. (2010). A model for glucose, insulin and beta-cell dynamics in subject with insulin resistance and patients with type 2 diabetes. *Journal of Clinical Pharmacology*, 50(8), 861-872.

Stenman, U.H. (2013). Standardization of hormone determinations. *Best Practical Research Clinical Endocrinology and Metabolism*, 27, 823-830.

Thamer, F.H. and Thamer, N. (2024). Gas Chromatography-Mass Spectrometry (GC-MS) profiling reveals newly described bioactive compounds in *Citrullus colocynthis*. *National Centre for Biotechnology Information*, 9(6), 781-790.

Turner, D.C., Schafer, M., Lacaster, S., Janmohamed, I., Gachanja, A. and Creasy, J. (2020). Gas Chromatography-Mass Spectrometry. *Royal Society of Chemistry*, ISBN-10, 1782629289.

Wu, A.H. and French, D. (2013). Implementation of liquid chromatography/mass spectrometry into the clinical laboratory. *Journal of Clinical Chemistry and Laboratory Medicine*, 420, 4-10.

Zeng, X., Li, S., Liu, L., Cai, S., Ye, Q., Xue, B., et al. (2023). Role of functional fatty acids in modulation of reproductive potential in Livestock. *Journal of Animal Science and Biotechnology*, 14, 24.