Scientia Africana, Vol. 23 (No. 3), August, 2024. Pp 47-58
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

CHARACTERIZATION OF THE PHYTOCHEMICAL CONSTITUENTS OF METHANOL EXTRACT OF *SPONDIAS MOMBIN STEM BARK USING* HIGH PERFORMANCE-LIQUID CHROMATOGRAPHY AND GAS CHROMATOGRAPHY-MASS SPECTROSCOPY

[1] Ikponmwosa-Eweka, O * and [2] Omoregie, E. S
[1]Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, BeninCity, Edo State, Nigeria
[2]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria
*Corresponding author: E-mail address: Omorede.aguebor@uniben.edu

Received: 17-04-2024
Accepted: 06-05-2024

https://dx.doi.org/10.4314/sa.v23i3.5

Journal Homepage: http://www.scientia-african.uniportjournal.info
Publisher: Faculty of Science, University of Port Harcourt.

ABSTRACT

Spondias mombin is commonly used in traditional medicine to treat various ailments. This study was aimed to analyze the bioactive components of the methanol extract of S. mombin stem by High-performance liquid chromatography (HPLC) and Gas Chromatography-Mass Spectroscopy (GC-MS). Quantitative analysis of the extract showed that it had appreciable quantities of flavonoids, phenols, tannins, and proanthocyanidins. The HPLC fingerprints of the extract also revealed the presence of flavonoids, tannins, saponins, alkaloids, other phenols, and steroids. The most prevalent flavonoids were the flavones, and the alkaloids present in high concentrations were ribalinidine and linamarin. Some other phenolics, such as resveratrol, ellagic acid, chlorogenic and pyrogallic, were in high concentration. The GC-MS data detected bioactive compounds; notably among them were 9,12 -octadecadienoic acid (linoleic acid), n-hexadecanoic acid, and 9-oxo nonanoic acid. This study has established the presence of bioactive chemicals in the methanol extract of S. mombin stem bark, and these phytochemicals could be responsible for the plant's medicinal properties.

Keywords: *Spondiasmombin*, bioactive, plant extract, HPLC, GC-MS

INTRODUCTION

Spondias mombin, a member of the Anacardiaceae family, is a tropical tree known for its diverse medicinal properties and traditional uses across various cultures. It is commonly referred to as hog plum or Spanish plum and locally referred to as *Ogheghe* (Edo), I*yeye*, *Akikan* (Yoruba), *Tsaadarmasar* (Hausa), and *Isikere* (Igbo) in Nigeria. *Spondias mombin* has garnered attention from researchers due to its extensive pharmacological potential. Among its parts, the stem bark stands out as a rich source of bioactive compounds, offering promising avenues for therapeutic exploration. Numerous studies have highlighted the pharmacological activities of *Spondias mombin* extracts, including antioxidant, anti-inflammatory, antimicrobial, and antidiabetic effects (Gomes *et al*., 2020; Santos *et al*., 2023; Oliveira *et al*., 2024). These beneficial properties have been attributed to phytochemicals and secondary metabolites synthesized by plants for defense and adaptation. However, a comprehensive

understanding of the phytochemical composition of *Spondias mombin* stem bark, particularly in methanol extracts, necessitates advanced analytical techniques.

High-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) are sophisticated analytical methods commonly employed to identify and quantify phytochemical constituents in plant extracts. HPLC offers high resolution and sensitivity, making it suitable for separating and analyzing complex mixtures of compounds. On the other hand, GC-MS provides structural information by coupling gas chromatography with mass spectrometry, facilitating the identification of individual compounds based on their mass spectra. In this context, the present study aimed to characterize the phytochemical constituents of the methanol extract of *Spondias mombin* stem bark using HPLC and GC-MS techniques. By employing these analytical tools, we sought to elucidate the chemical profile of the extract and identify its major bioactive constituents. This investigation is of significant importance for advancing our understanding of the medicinal properties of *Spondias mombin* and exploring its potential applications in the healthcare and pharmaceutical industries.

Previous research on *Spondias mombin* has demonstrated its pharmacological activities and highlighted the presence of various phytochemicals in different parts of the plant. For instance, studies have reported the presence of flavonoids, phenolic compounds, tannins, terpenoids, and alkaloids in *Spondias mombin* extracts, contributing to their antioxidant, anti-inflammatory, and antimicrobial properties (Cristofoli*et al*., 2019; Gomes *et al*., 2020; de Freitas*et al*., 2022). However, there is a paucity of literature specifically focusing on the phytochemical composition of the methanol extract of *Spondias mombin* stem bark using HPLC and GC-MS techniques. By filling this research gap, our study aimed to provide valuable insights into the chemical constituents of *Spondias mombin* stem bark, laying the foundation for further investigations into its therapeutic potential and supporting its utilization in traditional and modern medicine.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

The stem bark of *Spondias mombin* was collected from a farm in Benin City, Edo State. The plant was identified and authenticated in the Department of Plant Biology and Biotechnology, University of Benin, and the herbarium specimen was assigned voucher number UBH$_S$345. The stem bark was cut into pieces to dry under shade and pulverized. The pulverized stem bark was steeped in methanol for three days. The contents were stirred many times a day and filtered with Whatman filter paper at the end of the third day. The filtrate was evaporated to dryness with a rotary evaporator before being frozen with a freeze-dryer. The extract was weighed, placed in an airtight container, and refrigerated at 4^{o}C until use.

Quantitative phytochemical screening

Total phenolic and flavonoid contents were determined according to the Folin-Ciocalteu method by Cicco *et al*. (2009) and Miliauskas *et al*. (2004), respectively. At the same time, the total proanthocyanidin and total tannin contents of the methanol extract of the plant were carried out according to the method of Sun *et al*. (1998) and Polshettiwar *et al*. (2007) with some modifications.

Gas Chromatography-Mass Spectrometry Analysis (GC-MS) of the Extract

The extract was analyzed by GC-MS using a GC-MS-TQQQ apparatus equipped with an Agilent USB39375HHP-5MS column with capillary dimensions of 30 m × 250 m x 0.25 m. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. Injection fluid was used at a pressure of 10.97psi. After 0.5 min of equilibration, the oven was pre-heated to 70^{o}C for 5 min, then 10^{o}C/min. The run took 73 min in total. The MS transfer line was maintained

Scientia Africana, Vol. 23 (No. 3), August, 2024. Pp 47-58
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

at a temperature of 325^{o}C. The ionization method utilized was electron ionization at a source temperature of 250^{o} C and a potential of 70 eV. Total ion count (TIC) was utilized to assess the identification of compounds with a start mass of 20 amu and an end mass of 650 amu, and a scan duration of 200 ms. The compounds' spectra were matched to the NIST Reference Spectra Library database, with a Match Factor (MF) of 700 taken as adequate. The GC peak area was used to determine the relative percentage content of the detected chemicals.

High-Performance Liquid Chromatography (HPLC) of the Extract

Extraction of phytochemicals

Precisely 1 g of sample was weighed and added to a test tube along with 15 mL of ethanol and 10 mL of 50% m/v potassium hydroxide. The test tube was placed in a 600°C water bath for 60 min. The reaction product was transferred from the test tube to a separator funnel at the completion of the reaction period. Using 20 mL ethanol, 10 mL cold water, 10 mL hot water, and 3 mL acetonitrile, the tube was effectively washed. The combined extracts were washed three times with 10 mL of aqueous ethanol (10% v/v) solution. The solution was evaporated and dried with anhydrous sodium sulphate. Before transferring the sample to a vial for analysis, it was dissolved in 1000 uL of pyridine.

HPLC Analysis

Using a Buck 930 HPLC system (Waldbronn, Germany) equipped with a G1311C quaternary pump, a G1329B autosampler (0.1–100 L), a G1316A column oven (273–333 K), and a G1315D-DAD detector (190–950 nm), the monosaccharides tagged with PMP were analyzed. As the analytical column, a ShodexSHUGAR KS-802 column (4.6 mm, 150 mm, 5 m; Agilent) was utilized. The injection volume at 80°C was 20 L, and the eluant flow rate was 0.6 mL/min. The mobile phase A consisted of 100% acetonitrile, while the mobile phase B consisted of a mixture of distilled water and acetonitrile (90:10, v/v) in the presence of 0.045% KH_2PO_4-0.05% triethylamine buffer (pH 7.5); gradient elution was performed at 94-94-88-88% B with linear decreases at 0-4-5-20 min. At a wavelength of 245 nm, UV detection was assessed. The phytochemicals were identified by comparing the area and mass of an internal standard to the area of the discovered phytochemicals. The concentration of different phytochemicals was measured in micrograms per gramme.

RESULTS

Quantitative Phytochemical Constituents of Methanol Extract of *S. mombin* stem bark

Table 1 represents the total flavonoids, total phenols, total tannins, and total proanthocyanins of methanol extracts of *S. mombin* stem bark. The extract had appreciable amounts of these phytochemicals.

Table 1: Quantitative Phytochemical Constituents of methanol extract of *S. mombin*

Sample	Total Flavonoids (mg GAE/ g extract)	Total Phenols (mg QE/g extract)	Total Tannins (mgTAE/g extract)	Total Proanthocyanidins (mg CE / g extract)
S. mombin	41.86±0.58	204.0 ±8.4	97.8 ±9.96	69.1 ±1.3

Total flavonoid is expressed as mg quercertin equivalent (QE) / g extract; total phenol is expressed as mg gallic acid equivalent (GAE) / g extract; total tannins is expressed as mg tannic acid equivalent (TAE)/g extract; proanthocyanidin content is expressed as mg catechin equivalent (CE) / g extract. Values are expressed as mean ± SEM; n = 3.

High-Performance Liquid Chromatography of the methanol extract of *S. mombin* stem bark

Figure 1 and Table 2 illustrate the outcomes of the HPLC fingerprint analysis of the methanol extract of *S. mombin* stem bark. The result reveals that *S. mombin* extract contain flavonoids, tannins, saponins, alkaloids, phenolics, and steroids. Flavone, a flavonoid is observed to have the highest concentration (21.19 µg/mL). This is followed by pyrogallic acid (19.27 µg/mL) and ribalinidine, an alkaloid (16.83 µg/mL).

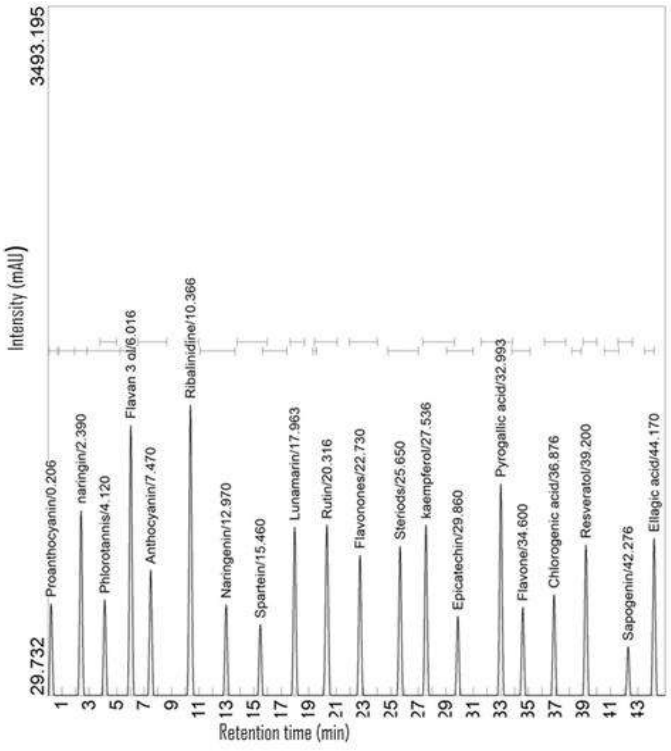


Figure 1: HPLC chromatogram of the methanol extract of *S. mombin stem bark*

Table 2: HPLC Fingerprint of Phytochemicals from Methanol Extract of *S. mombin* Stem Bark

Type of Phytochemical	Phytochemical	Concentration (µg/mL)
Flavonoids	Proanthocyanin	8.58
	Anthocyanin	10.92
	Naringin	15.46
	Flavonones	16.44
	Flavone	21.19
	Rutin	15.84
	Kaempferol	7.99

Scientia Africana, Vol. 23 (No. 3), August, 2024. Pp 47-58
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

	Epicatechin	8.23
	Naringenin	2.64
Tannins	Phlorotannins	4.06
Saponins	Sapogenin	5.77
Alkaloids	Ribalinidine	16.83
	Lunamarin	16.62
	Spartein	8.92
Other Phenolics	Resveratrol	7.78
	Ellagic acid	6.12
	Chlorogenic acid	11.05
	pyrogallic acid	19.27
Steroids	Steroids	17.30

Gas Chromatography-Mass spectroscopy of the methanol extract of *S. mombin* stem bark

The GC-MS analysis of the methanol extracts of *S. mombin* stem bark showing the compound name, retention time, molecular weight (g/mol), structural formula and the structure of the compounds of some of the identified compounds are presented in Table 3. Also, Figures 2 depict the chromatograms of *S. mombin* with their peak areas.

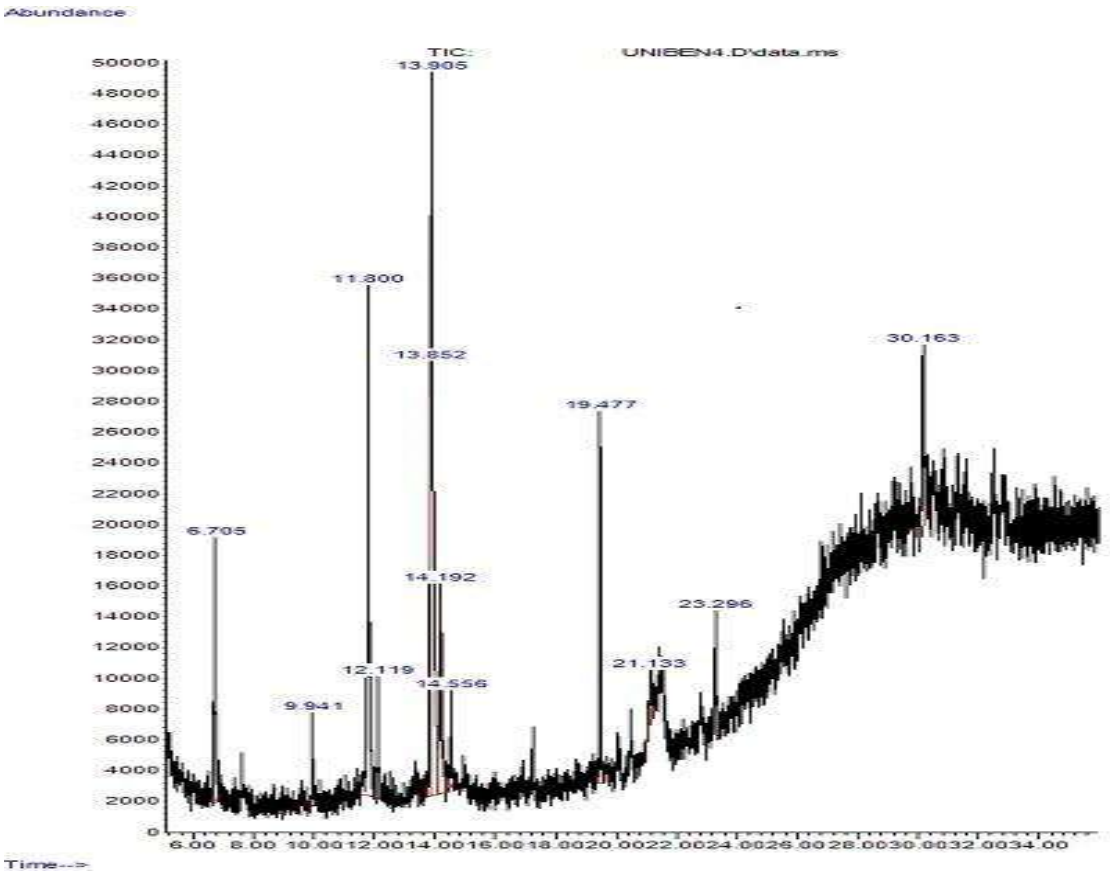
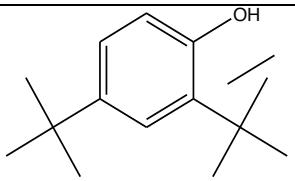
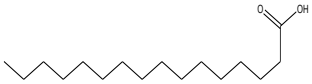
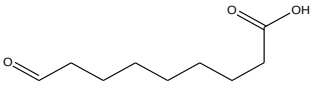
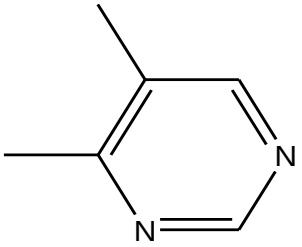
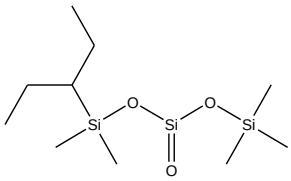
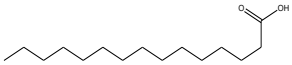
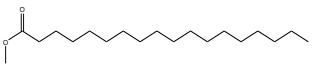
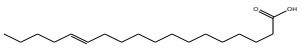


Figure 2: **GC-MS chromatogram of methanol extract of *S. mombin* (stem bark)**

Table 3: List of identified phytocompounds from the GC-MS fingerprint

Name of Compound	Formula	MW	RT(Min)	Area %	Chemical structure
2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	6.07	7.05	
Tridecanoic acid	$C_{13}H_{26}O_2$	214.3	11.80	17.47	
n-Hexadecanoic acid	$C_6H_{32}O_2$	256	11.80	17.47	
9- Oxononanoic acid	$C_9H_{16}O_3$	172.0	15.01	4.83	

Scientia Africana, Vol. 23 (No. 3), August, 2024. Pp 47-58
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

9,12-Octadecadienoic acid (linoleic acid)	$C_{18}H_{32}O_2$	280.5	13.852	9.28	
Pyrimidine, 4,5-dimethyl	$C_6H_8N_2$	108.0	23.21	4.75	
Silicic acid, diethylbis (trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	296.0	29.55	4.68	
Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4	11.8	17.47	
Methyl stearate	$C_{19}H_{38}O$	298.5	30.16	7.57	
Trans-13-octadecenoic acid	$C_{18}H_{34}O_2$	282.5	23.29	3.11	

DISCUSSION

Phytochemicals are essential as protective and disease-fighting molecules that aid the body in preventing or combating disease and are, therefore, necessary for human survival (Forni *et al*., 2019). Their therapeutic uses in the prevention and treatment of various ailments are the basis for their widespread usage in traditional and ethnomedicinal practices in various cultures. Ascertaining the phytochemical constituents and antioxidant status of medicinal plants has become an excellent point at which to commence research on knowing their therapeutic potential. The therapeutic benefits of plants may be attributed to a single component or synergy of phytoconstituents (Pezzani *et al*., 2019). The presence of an appreciable quantity of therapeutically active chemicals, including flavonoids, tannins, phenols, and proanthocyanidins, was discovered in methanol extracts of *S. mombin* stem bark in Table 1.

The HPLC fingerprint of the methanol extract of *S. mombin* stem bark revealed the presence of fifteen (15) compounds, which include flavonoids, tannins, saponins, alkaloids, other phenols, and steroids. The most prevalent flavonoids were flavone, rutin, flavanones, naringin, anthocyanin, epicatechin, proanthocyanin, and naringenin. The alkaloids present in high concentrations were ribalindine and linamarin. Some other phenolics, such as resveratrol, ellagic acid, chlorogenic and pyrogallic, were in high concentration.De Freitas *et al*. (2022) and Akinmoladun*et al*. 2021) identified a similar pattern of metabolites to those found in this work although catechin, caffeic acid and quercetin present in their research where absent in this study. The differences in the chemical composition observed in these studies can be justified due to a series of experimental factors such as the polarity of the solvent, the plant part used as well as due to intrinsic (genetic) and extrinsic (environmental) factors (Bezerra *et al*. 2017). Additionally, HPLC analyses performed by Omoboyowa*et al*. (2023)

demonstrated that *S. mombin* stem bark had rutin, chlorogenic acid, epicatechin, and kaemferol as significant constituents, corroborating the findings of this present research.

Flavonoids consist of natural substances with variable phenolic groups, mainly in vegetables and some grains, stems, and flowers. They are well known for their valuable health benefits, mainly their attributable anti-oxidative, anti-mutagenic, anti-inflammatory, anti-carcinogenic properties, and enzyme modulatory functions (Panche *et al*.,2016). In the present study, the flavonoids identified include proanthocyanin, anthocyanin, naringin, flavonones, flavone, rutin, kaempferol, epicatechin, and naringenin. Flavone had the highest concentration, while naringenin was of the lowest concentration (Table 2). The rich flavonoid content of the methanol extract of *S. mombin* stem bark confers on it many pharmacological activities such as anti-inflammatory, antipyretic, hypoglycemic, antifungal, antibacterial, and anti-tumor and wound healing properties.

Tannins are primarily water-soluble polyphenols that are present in a variety of plant foods. They are found in tea, cocoa, vegetables, legumes, and some unripe fruit (Sharma *et al*.,2019). *S. mombin* stem bark contained low tannins. Tannins have played a key role in Asian traditional medicine, where plant extracts containing tannins are used as an astringent and a diuretic. They have also been used to treat diarrhea, gastrointestinal ulcers, and tumors. They also possess anti-inflammation and antioxidant activities (Ghosh, 2015). On the other hand, Saponins comprise a group of structurally related naturally occurring compounds containing either a steroid or triterpenoid aglycone (sapogenin) found mostly in plants and other lower marine animals, including some bacteria. Some of the pharmacological effects ascribed to saponins include immunomodulatory, anti-inflammatory, anti-fungal, antiviral, antibacterial, hypercholesterolaemic, and anti-carcinogenic properties (Francis *et al*., 2005), thus making them very essential in human and animal nutrition. Plant alkaloids remain one of the largest groups of natural products made up of structurally of diverse and biogenetically unrelated molecules. They possess a wide range of pharmacological activities and have been used as a component of many herbal remedies (Alves de Almeida *et al*., 2017). They include narcotics, analgesics, morphine, and codeine. Also, they have been shown to possess potent antimalarial, antimicrobial, and antiprotozoal properties (Franck *et al*.,2004). The result of the present study showed that the methanol extract of *S. mombin* stem bark contains a significant amount of alkaloids, with ribalinidine having the highest concentration, followed by linamarin and then sparteine (Table 2). Lunamarin and ribalinidine have been reported to have radical scavenging functions. Also, lunamarin possesses anticancer, immunomodulatory, anti-estrogenic, and anti-amoebic properties (Ugoeze*et al*.,2020). These alkaloid contents could be attributed to some of the pharmacologic properties of the extract of *S. mombin* stem bark. Several other phytochemicals, such as resveratrol, chlorogenic acid, and steroids, were also determined.

Phytoconstituents contained in the plant as revealed in this study via the GC-MS profiling of the methanol extract of *S. mombin* stem bark (Table 3 and Figure 2) indicates a rich array of bioactive compounds, some of which have diverse pharmacological potentials. Notable among them was linoleic acid in *S. mombin*, a precursor of arachidonic acid, the primary unsaturated 20-carbon fatty acid required to produce prostaglandins (PGs) via the cyclooxygenase pathway. PGs are crucial for preserving the integrity of the gastroduodenal mucosa by promoting mucosal bicarbonate secretion, accelerating cell proliferation, and improving mucus secretion and mucosal blood flow (Brozozoswki *et al*., 2005). Additionally, they enhance lysosomal stability and mucosal phospholipid synthesis. Thus, a higher intake of linoleic acid may increase the generation of

Scientia Africana, Vol. 23 (No. 3), August, 2024. Pp 47-58
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

endogenous PGs, promoting gastric health (Pagkalos *et al*., 2009).Orunmwensodia*et al*. (2020) study on the GC-MS profiling of the oily hexane:ethylacetate fraction of *S. mombin* reported similar compounds in the fractions.

CONCLUSION

This study has established the presence of bioactive chemicals in the methanol extract of *S. mombin* stem bark, and these phytochemicals could be responsible for the plant's medicinal properties.

Funding

This Research was funded by the Nigeria Tertiary Education Trust Fund (TETfund) Institutional Based Research Fund (IBR) with reference code TETF/DR&D/CE/UNI/EDO/ IBR/2023/VOL.III, NO 5.

REFERENCE

Akinmoladun, A.C., Adelabu, A.A., Saliu, I.O., Adetuyi, A.R. and Olaleye, M.T. (2021). Protective properties of Spondias mombin Linn leaves on redox status, cholinergic dysfunction and electrolyte disturbance in cyanide-intoxicated rats. *Science progress*, *104*(2), p.00368504211011866.

Alves de Almeida, A.C., de-Faria, F.M., Dunder, R.J., Manzo, L.P.B., Souza-Brito, A.R.M. and Luiz-Ferreira, A. (2017). Recent trends in pharmacological activity of alkaloids in animal colitis: potential use for inflammatory bowel disease. *Evidence-based complementary and alternative medicine*,3. 45-62

Brzozowski, T., Konturek, P.C., Konturek, S.J., Brzozowska, I. and Pawlik, T. (2005). Role of prostaglandins in gastroprotection and gastric adaptation. *Journal of physiology and pharmacology*, *56*(5), pp.33-55.

Chao, C.L., Weng, C.S., Chang, N.C., Lin, J.S., Kao, S.T. and Ho, F.M. (2010). Naringenin more effectively inhibits inducible nitric oxide synthase and cyclooxygenase-2 expression in macrophages than in microglia. *Nutrition research*, *30*(12), pp.858-864.

Chen, A.Y. and Chen, Y.C. (2013). A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention. *Food chemistry*, *138*(4), pp.2099-2107.

Cicco, N., Lanorte, M.T., Paraggio, M., Viggiano, M. and Lattanzio, V. (2009). A reproducible, rapid and inexpensive Folin–Ciocalteu micro-method in determining phenolics of plant methanol extracts. *Microchemical journal*, *91*(1), pp.107-110.

Cristofoli, N.L., Lima, C.A.R., Vieira, M.M.C., Andrade, K.S. and Ferreira, S.R. (2019). Antioxidant and antimicrobial potential of cajazeira leaves (Spondiasmombin) extracts. *Separation Science and Technology*, *54*(4), pp.580-590.

de Freitas, M.A., da Cruz, R.P., Dos Santos, A.T.L., Almeida-Bezerra, J.W., Machado, A.J.T., Dos Santos, J.F.S., Rocha, J.E., Boligon, A.A., Bezerra, C.F., de Freitas, T.S. and do Nascimento Silva, M.K. (2022). HPLC–DAD analysis and antimicrobial activities of Spondias mombin L.(Anacardiaceae). *3 Biotech*, *12*(3), p.61.

Del Rio, D., Rodriguez-Mateos, A., Spencer, J.P., Tognolini, M., Borges, G. and Crozier, A. (2013). Dietary (poly) phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants & redox signaling*, *18*(14), pp.1818-1892.

Forni, C., Facchiano, F., Bartoli, M., Pieretti, S., Facchiano, A., D'Arcangelo, D., Norelli, S., Valle, G., Nisini, R., Beninati, S. and Tabolacci, C. (2019). Beneficial role of phytochemicals on oxidative stress and age-related diseases. *BioMed research international*, **8**:78-82

Francis, G., Kerem, Z., Makkar, H.P. and Becker, K. (2002). The biological action of saponins in animal systems: a review. *British journal of Nutrition*, *88*(6), pp.587-605.

Franck, X., Fournet, A., Prina, E., Mahieux, R., Hocquemiller, R. and Figadère, B. (2004). Biological evaluation of substituted quinolines. *Bioorganic & medicinal chemistry letters*, *14*(14), pp.3635-3638.

Ganeshpurkar, A. and Saluja, A.K.(2017). The pharmacological potential of rutin. *Saudi pharmaceutical journal*, *25*(2), pp.149-164.

Ghosh, D. (2015). Tannins from foods to combat diseases. *Int J Pharm Res Rev*, *4*(5), pp.40-44.

Gomes, M.S., Lins, R.D.A.U., Langassner, S.M.Z., da Silveira, É.J.D., de Carvalho, T.G., de Sousa Lopes, M.L.D., de Souza Araújo, L., de Medeiros, C.A.C.X., de Carvalho Leitao, R.F., Guerra, G.C.B. and de Araújo Junior, R.F. (2020). Anti-inflammatory and antioxidant activity of hydroethanolic extract of *Spondias mombin* leaf in an oral mucositis experimental model. *Archives of Oral Biology*, *111*, p.104664.

Jiang, N., Doseff, A.I. and Grotewold, E. (2016). Flavones: from biosynthesis to health benefits. *Plants*, *5*(2), p.27.

Liu, X., Wang, W., Hu, H., Tang, N., Zhang, C., Liang, W. and Wang, M. (2006). Smad3 specific inhibitor, naringenin, decreases the expression of extracellular matrix induced by TGF-β1 in cultured rat hepatic stellate cells. *Pharmaceutical research*, *23*, pp.82-89.

Miliauskas, G., Venskutonis, P.R. and Van Beek, T.A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food chemistry*, *85*(2), pp.231-237.

Mulvihill, E.E., Allister, E.M., Sutherland, B.G., Telford, D.E., Sawyez, C.G., Edwards, J.Y., Markle, J.M., Hegele, R.A. and Huff, M.W. (2009). Naringenin prevents dyslipidemia, apolipoprotein B overproduction, and hyperinsulinemia in LDL receptor–null mice with diet-induced insulin resistance. *Diabetes*, *58*(10), pp.2198-2210.

Oladimeji, A.V. and Valan, M.F. (2020). HPLC techniques for phytochemistry. *International Journal of Chemical Studies*, *8*(6), pp.2590-2596.

Oliveira, F.C.D.M., Holanda, T.M.V., de Assis, C.F., Xavier Júnior, F.H. and de Sousa Júnior, F.C. (2024). Flours from Spondias mombin and Spondias tuberosa seeds: Physicochemical characterization, technological properties, and antioxidant, antibacterial, and antidiabetic activities. *Journal of Food Science*, *89*(1), pp.342-355.

Omoboyowa, D.A., Agoi, M.D., Shodehinde, S.A., Saibu, O.A. and Saliu, J.A. (2023). Antidiabetes study of Spondias mombin (Linn) stem bark fractions in high-sucrose diet-induced diabetes in Drosophila melanogaster. *Journal of Taibah University Medical Sciences*, *18*(4), pp.663-675.

Oršolić, N., Gajski, G., Garaj-Vrhovac, V., Đikić, D., Prskalo, Z.Š. and Sirovina, D. (2011). DNA-protective effects of quercetin or naringenin in alloxan-induced diabetic mice. *European journal of pharmacology*, *656*(1-3), pp.110-118.

Orumwensodia, K.O., Uadia, P.O. and Choudhary, M.I. (2021). Phytotoxicity, cytotoxicity and chemical composition of Spondias mombin Linn. Stem bark. *Clinical Phytoscience*, *7*, pp.1-9.

Pagkalos, V.A., Moschandreas, J., Kiriakakis, M., Roussomoustakaki, M., Kafatos, A. and Kouroumalis, E. (2009). Fatty acid composition of subcutaneous adipose tissue and gastric mucosa: is there a relation with gastric ulceration? *BMC gastroenterology*, *9*(1):1-9.

Panche, A.N., Diwan, A.D. and Chandra, S.R. (2016). Flavonoids: an overview. *Journal of nutritional science*, *5*, p.e47.

Patel, K., Singh, G.K. and Patel, D.K. (2018). A review on pharmacological and analytical aspects of naringenin. *Chinese journal of integrative medicine*, *24*, pp.551-560.

Pezzani, R., Salehi, B., Vitalini, S., Iriti, M., Zuñiga, F.A., Sharifi-Rad, J., Martorell,

Scientia Africana, Vol. 23 (No. 3), August, 2024. Pp 47-58
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

M. and Martins, N. (2019). Synergistic effects of plant derivatives and conventional chemotherapeutic agents: an update on the cancer perspective. *Medicina*, *55*(4), p.110.

Polshettiwar, S.A., Ganjiwale, R.O., Wadher, S.J. and Yeole, P.G. (2007). Spectrophotometric estimation of total tannins in some ayurvedic eye drops. *Indian Journal of Pharmaceutical Sciences*, *69*(4).

Rodrigues, K.F., Hesse, M. and Werner, C. (2000). Antimicrobial activities of secondary metabolites produced by endophytic fungi from Spondias mombin. *Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms*, *40*(4), pp.261-267.

Saigo, T., Wang, T., Watanabe, M. and Tohge, T. (2020). Diversity of anthocyanin and proanthocyanin biosynthesis in land plants. *Current opinion in plant biology*, *55*, pp.93-99.

Santos, É.M.D., Ataide, J.A., Coco, J.C., Fava, A.L.M., Silvério, L.A.L., Sueiro, A.C., Silva, J.R.A., Lopes, A.M., Paiva-Santos, A.C. and Mazzola, P.G. (2023). Spondiassp: shedding light on its vast pharmaceutical potential. *Molecules*, *28*(4), p.1862.

Sharma, K., Kumar, V., Kaur, J., Tanwar, B., Goyal, A., Sharma, R., Gat, Y. and Kumar, A. (2021). Health effects, sources, utilization and safety of tannins: A critical review. *Toxin Reviews*, *40*(4), pp.432-444.

Sun, B., Ricardo-da-Silva, J.M. and Spranger, I. (1998). Critical factors of vanillin assay for catechins and proanthocyanidins. *Journal of agricultural and food chemistry*, *46*(10), pp.4267-4274.

Symonowicz, M. and Kolanek, M. (2012). Flavonoids and their properties to form chelate complexes. *Biotechnology and Food Science*, *76*(1), pp.35-41.

Ugoeze, K.C., Oluigbo, K.E. and Chinko, B.C. (2020). Phytomedicinal and Nutraceutical Benefits of the GC-FID Quantified Phytocomponents of the Aqueous Extract of *Azadirachtaindica* leaves. *Journal of Pharmacy and Pharmacology Research*, *4*(4), pp.149-163.