www.ajbrui.org

Afr. J. Biomed. Res. Vol. 25 (January, 2022); 101 - 106

Research Article

Phytochemical, Antimicrobial and Toxicity Assessment of *Dacryodes edulis* (G. Don.) H. J. Lam Leaf Extracts.

***Aponjolosun B. S. and Fasola T. R.**

Department of Botany, University of Ibadan. Ibadan, Nigeria

ABSTRACT

The leaf of *Dacryodes edulis* is traditionally used for dressing bruises and wounds; treating diarrhea, dysentery, toothache, gum problems, and tonsillitis. Some of these uses have not been screened scientifically to know its efficacy and safety in consumption. Therefore, this research focused on antimicrobial ability of *D. edulis* leaf on some selected microorganisms and its toxicity evaluation. The leaf of *D. edulis* was extracted with methanol and successively partitioned with hexane, ethyl acetate, butanol, and water. Each of the extracts was screened for phytochemical constituents using standard methods. They were also tested on twelve microorganisms using standard techniques. Based on performance, acute toxicity test of extracts was conducted on male Wistar rats following standard procedures. Their excised kidneys and livers were histologically examined. Both the leaf methanol extract and fractions contained tannins, anthraquinones, steroids, terpenoids, saponins, alkaloids, phenolics, flavonoids, carotenoids, and cardiac glycosides. The ethyl acetate fraction showed significant zones of inhibition for *Klebsiella pneumoniae*, *Bacillus cereus, Staphylococcus aureus, Serratia marcescens* (29, 28, 25, 20 mm, respectively), while the water fraction had 20 mm for *Pseudomonas aeruginosa*. Some fractions of the leaf also inhibited *Trichophyton metagrophytes, Cladosporium herbarum* and *Trichoderma* species. None of the rats died during the study and the histology of their livers and kidneys showed no lesions at 2900 mg/kg dose. The phytochemical constituents and antibacterial effectiveness of *D. edulis* leaf extracts supported some traditional uses, while non-toxicity of the leaf extracts showed their safety for use.

Keywords: *Herbal medicine, Secondary metabolites, Anti-bacterial activity, Anti-fungal activity, Histology*

*Author for correspondence: Email: *prosperbas@gmail.com* *Tel: +234 8086776323*

Received: September 2020; Accepted: August 2021

INTRODUCTION

Therapeutic plants are prospective sources of novel drugs and as alternative remedies for different health problems (Sidhu and Sharma, 2013). Phytochemicals are bioactive non-essential nutrients of plants (Elnour *et al* . 2018). However, the use of phytochemicals has been considered to be safer and congenial to the biology of the human body (El Aziz *et al*., 2019).

Dacryodes edulis (G. Don.) H. J. Lam commonly called African plum or African pear belongs to the family Burseraceae. It is an evergreen tree attaining a height of 18 – 40 m. It has a relatively short trunk and a deep, dense crown. The bark is pale gray and rough with droplets of resin. The leaves are compound with 5 – 8 pairs of leaflets. The upper surface of the leaves is glossy. The fruit is an ellipsoidal drupe which varies in length from 4 – 12 cm. The skin of the fruit is dark blue or violet, whereas the flesh is pale to light green. The bark, leaf, fruit and resin of *D. edulis* are reported as antimalaria, anthelmintics, astringent, for treating wounds, elephantiasis curative, skin treatment, and in clearing pregnancy stretch marks (Burkill, 2004; Orwa *et al* ., 2009; Omonhinmin, 2014). The problem of high cost and unavailability of some modern drugs are making many people in the developing nations to use alternative therapy. However, some challenges of toxicity from herbal drug abuse cannot be overlooked. There is therefore the need to investigate on *D. edulis* for antimicrobial properties and toxicity.

MATERIALS AND METHODS

Collection and preparation of the plant: *Dacryodes edulis* leaves were collected at Abadina, University of Ibadan, authenticated with the voucher number UIH 22488 at the University of Ibadan Herbarium (UIH), Ibadan. The leaves were thoroughly washed with clean water, air dried for twelve days and milled. The plant (1 kg) was macerated for forty-eight hours with three litres of methanol (BDH, England). The liquid extract was decanted, filtered with Whatman filter paper No. 1; concentrated with rotary evaporator and freeze dried. The methanol extract was then successively fractionated using analytical grade solvents: n-hexane, ethyl acetate, butanol, and distilled water. Each fraction was concentrated with rotary evaporator and freeze dried for use.

Phytochemical assays: The phytochemical assays were carried out on each of the methanol extract and fractions of hexane, ethyl acetate, butanol, aqueous, and pulverized *D. edulis* leaves. Saponins, tannins, alkaloids, phenolics, anthraquinones, flavonoids, steroids, terpenoids, cardiac glycosides, and carotenoids were quantitatively screened following Marcano and Hasenawa (1991), Harborne (1998), and Mayuri (2012) methods.

Antimicrobial assays: Two typed strains of Gram-positive bacteria: *Staphylococcus aureus* (NCIB 8588), *Bacillus cereus* (NCIB 6349); four typed strains of Gram-negative bacteria: *Serratia marcescens* (NCIB 1377), *Klebsiella pneumoniae* (NCIB 418), *Pseudomonas aeruginosa* (NCIB 950), and *Proteus vulgaris* (NCIB 67); and six molds: *Trichophyton metagrophytes*, *Cladosporium herbarum, Trichoderma species, Penicillium camemberti, Aspergillus flavus and Rhizopus stolonifer* were used for this study. Thirty-eight grams of Muller Hinton Agar and 48 g of Malt Extract Agar were separately dissolved in one litre of distilled water following manufacturers' instructions. They were boiled and 20 mL was dispensed into each MacCartney bottle, autoclaved at 121 0C, 15 psi (1 kg/cm3) pressure for fifteen minutes and kept in a molten state. Agar well diffusion method was employed for the antibacterial test (Arekemase *et al*., 2011), while Ditch plate method was used for the antifungal test (Sodha *et al*., 2015).

Toxicity experiment: Seventy (70) male Wistar rats were procured from the Central Animal House, University of Ibadan, grouped into five per cage and given food and water *ad libitum* for seventy-two hours for acclimatization. Different doses of 10, 100, 1000, 1600, 2900 and 5000 mg/kg ethyl acetate and the aqueous fractions of *D. edulis* leaf using a dissolvent - 20 % Dimethyl sulphoxide (DMSO) were prepared. The doses were orally administered to the animals via cannulas and syringes. A group was given 20 % DMSO only (control I), while another group was not treated (control II). The animals were fasted and observed for twenty-four hours before sacrificed by quick cervical dislocation. The rats' livers and kidneys were excised, preserved in 10 % formalin, sliced, stained and examined under microscope according to Lorke (1983).

RESULTS

Phytochemical constituents of *Dacryodes edulis* leaf: All the phytochemical constituents screened were present in the leaf powder, methanol extract and all the fractions of *D. edulis* . However, the quantity varied with sample. As an instance, the hexane fraction had the highest saponins value in all, while ethyl acetate fraction was best for tannins. Methanol extract was highest in alkaloids, while hexane fraction for phenolics. Butanol fraction had the highest steroids, anthraquinones, terpenoids and carotenoids, as the pulverized leaf was highest in flavonoids, while methanol extract was highest in cardiac glycosides (Table 1).

Antibacterial activities of *Dacryodes edulis* leaf: The zones of inhibition increase with increase in concentration of the methanol extract and all the fractions. However, at the highest concentration (100 mg/mL), the ethyl acetate fraction had the highest zones of inhibition for *K. pneumoniae, B. cereus, S. aureus and S. marcescens* (29, 28, 25 and 20 mm, respectively). Moreover, ethyl acetate and hexane fractions had the same inhibition for *P. vulgaris* (16 mm), while water fraction was highest for *P. aeruginosa* (20 mm) (Table 2).

Table 1:
Phytochemical constituents of *Dacryodes edulis* leaf

Parameters	Leaf extract and fractions					
	M	H	E	B	A	J
Saponins (mg/100g)	$571.67 \pm 7.26_c$	$1628.33 \pm 18.78_a$	$636.67 \pm 9.28_b$	$13.33 \pm 1.67_f$	$358.33 \pm 7.26_d$	$231.67 \pm 7.26_e$
Tannins (mg/100g)	$76.67 \pm 4.41_d$	$91.67 \pm 1.67_c$	$1111.67 \pm 9.28_a$	$168.33 \pm 6.01_b$	$13.33 \pm 1.67_f$	$50.00 \pm 2.87_e$
Alkaloids (mg/100g)	$961.67 \pm 7.26_a$	$416.67 \pm 6.01_f$	$808.33 \pm 4.41_c$	$630.00 \pm 10.00_d$	$480.00 \pm 10.41_e$	$916.67 \pm 10.14_b$
Phenolics (GAE/g)	$26.67 \pm 0.15_d$	$92.33 \pm 0.15_a$	$62.27 \pm 0.15_c$	$79.47 \pm 0.12_b$	$25.33 \pm 0.09_e$	$8.57 \pm 0.15_f$
Anthraquinones (mg/100g)	$435.00 \pm 7.64_c$	$235.00 \pm 10.41_d$	$578.33 \pm 9.28_b$	$831.67 \pm 10.14_a$	$165.00 \pm 8.66_e$	$116.67 \pm 6.01_f$
Flavonoids (mg/100g)	$355.00 \pm 8.66_d$	$183.33 \pm 3.33_f$	$855.00 \pm 7.64_c$	$1435.00 \pm 10.41_b$	$230.00 \pm 7.64_e$	$1505.00 \pm 10.41_a$
Steroids (mg/100g)	$551.67 \pm 6.01_b$	$345.00 \pm 8.66_e$	$650.00 \pm 7.64_a$	$673.33 \pm 7.26_a$	$381.67 \pm 10.93_d$	$418.33 \pm 4.41_c$
Terpenoids (mg/100g)	$471.67 \pm 4.41_d$	$525.00 \pm 7.64_c$	$381.67 \pm 6.01_e$	$948.33 \pm 10.14_a$	$670.00 \pm 7.64_b$	$81.67 \pm 4.41_f$
Cardiac glycosides (mg/100g)	$33.33 \pm 1.67_a$	$20.00 \pm 0.00_b$	$13.33 \pm 1.67_c$	$11.67 \pm 1.67_d$	$20.00 \pm 2.89_b$	ND
Carotenoids (µg/100g)	$1083.33 \pm 7.26_d$	$1535.00 \pm 8.66_c$	$1718.33 \pm 10.14_b$	$2141.67 \pm 6.01_a$	$1726.67 \pm 6.01_b$	$720.00 \pm 2.89_e$

*n = 3; Values = Means ± S.E. Subscripts are level of significance (p < 0.05) for rows. M = Methanol extract of D. edulis , H = Hexane fraction of D. edulis , E = Ethyl acetate fraction of D. edulis , B = Butanol fraction of D. edulis , A = Aqueous fraction of D. edulis , J = pulverized leaf (control) of D. edulis . ND = Not detected. *1000 µg = 1mg*

.

Table 2:
Antibacterial activities of *Dacryodes edulis* leaf extracts

Microorganism	M, E, H, W concentration (mg/mL)					
	3.125	6.25	12.5	25	50	100
	Zones of inhibition (mm)					
Staphylococcus aureus (NCIB 8588)	E (12) M (10)	E (15) M (12)	E (16) M (15)	E (18) M (16)	E (20) M (18)	E (25) M (21)
Serratia marcescens (NCIB 1377)	-	-	E (9)	E (11)	E (15)	E (20)
Bacillus cereus (NCIB 6349)	E (10)	E (17) M (12)	E (20) M (13)	E (22) M (16)	E (25) M (18) H (10)	E (28) M (22) H (12)
Klebsiella pneumoniae (NCIB 418)	E (9)	E (17) M (11)	E (23) M (13)	E (25) M (15)	E (27) M (17) H (9)	E (29) M (21) H (10)
Proteus vulgaris (NCIB 67)	-	-	-	E (9)	E (14) H (10)	E (16) H (16)
Pseudomonas aeruginosa (NCIB 950)	-	-	W (15)	W (16) E (10)	W (18) E (12)	W (20) E (17)

M = methanol extract, H = hexane fraction, E = ethyl acetate fraction, W = aqueous fraction, - = No inhibition, NCIB = National Collection of Industrial Bacteria, Scotland.

Table 3:
Antifungal activities of *Dacryodes edulis* leaf extracts

Microorganism	M, E, H, W concentration (mg/mL)					
	3.125	6.25	12.5	25	50	100
	Zones of inhibition (mm)					
Penicillium camemberti	-	-	-	-	-	-
Trichophyton metagrophytes	-	-	-	E (19	E (26)	E (54)
Aspergillus flavus	-	-	-	-	-	-
Rhizopus stolonifer	-	-	-	-	-	-
Trichoderma sp.	-	-	-	-	-	E (19)
Cladosporium herbarum	- -	- -	- -	- E(18)	- E (30)	W (47) E (44)

M = methanol extract, H = hexane fraction, E = ethyl acetate fraction, W = aqueous fraction, - = No inhibition, NCIB = National Collection of Industrial Bacteria, Scotland.

Antifungal activities of *Dacryodes edulis* leaf: In all the samples, activities were not observed at lower concentrations below 25 mg/mL. The best zone of inhibition was found in ethyl acetate fraction against *T. metagrophytes*, followed by water fraction against *C. herbarum* at the same concentration. However, methanol extract and hexane fraction were inactive (Table 3).

Toxicity of *Dacryodes edulis* in rats: None of the rats died during the experiment, showing that the ethyl acetate and aqueous fractions of *D. edulis* leaf were not toxic. The photomicrographs of the livers and kidneys for *D. edulis* are shown in Plates 1 – 4. There were no visible lesions on the livers (except at 5000 mg/kg for ethyl acetate fraction) and kidneys of the rats.

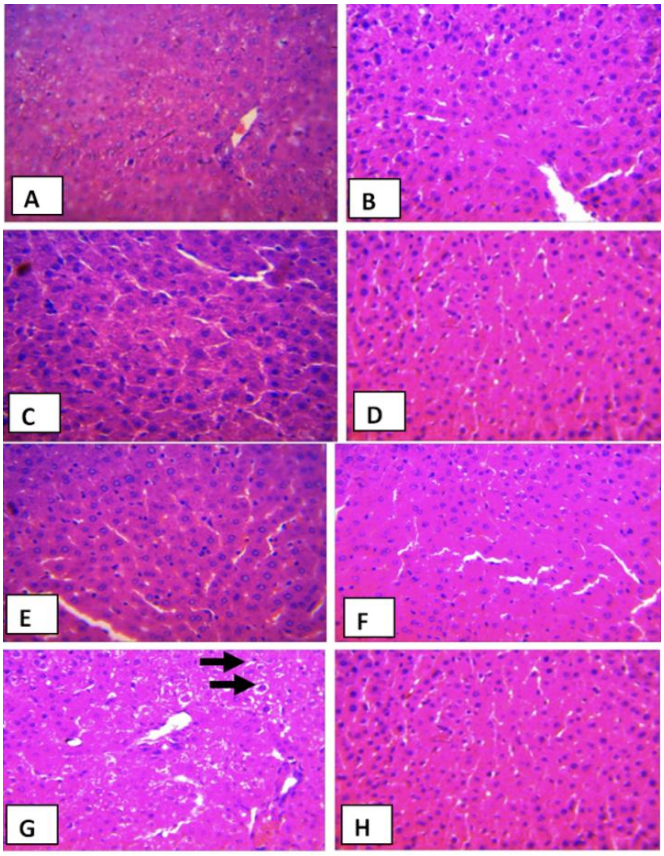

Plate 1:
Liver histology (x400) of rats given ethyl acetate fraction of *Dacryodes edulis* (De) leaf
A = rat given 10 mg/kg of De; B = rat given 100 mg/kg of De; C = rat given 1000 mg/kg of De; D = rat given 20 % Dimethyl sulphoxide (control I); E = rat given 1600 mg/kg of De; F = rat given 2900 mg/kg of De; G = rat given 5000 mg/kg of De: showing mild vacuolar degeneration of hepatocytes (arrows); H = rat without treatment (control II). All (except G) show no visible lesions

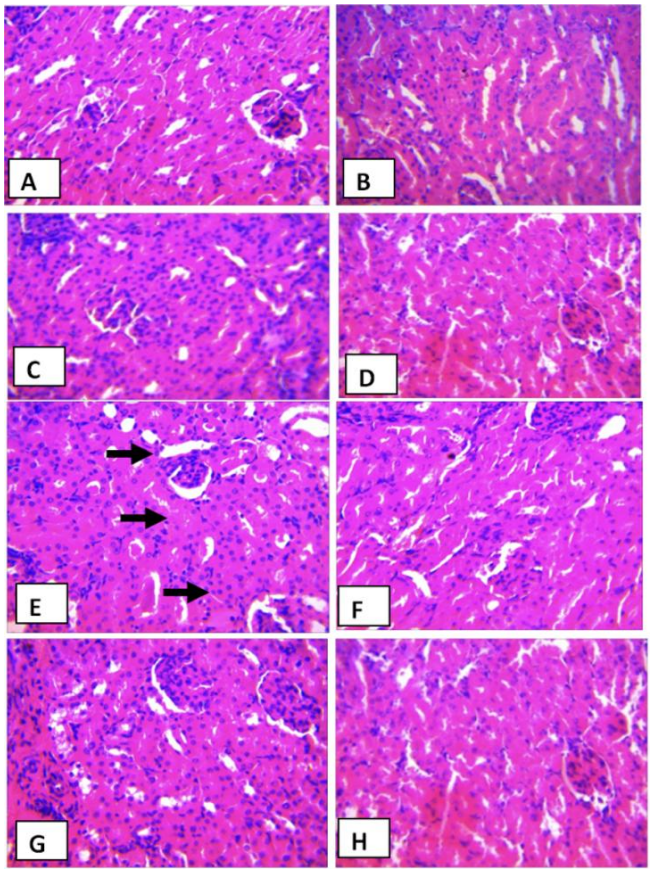

Plate 2
Kidney histology (x400) of rats given ethyl acetate fraction of *Dacryodes edulis* (De) leaf
A = rat given 10 mg/kg of De; B = rat given 100 mg/kg of De; C = rat given 1000 mg/kg of De; D = rat given 20 % Dimethyl sulphoxide (control I); E = rat given 1600 mg/kg of De: showing pink staining casts in the tubular lumen of the renal tubules (arrows); F = rat given 2900 mg/kg of De; G = rat given 5000 mg/kg of De; H = rat without treatment (control II). All show no visible lesions

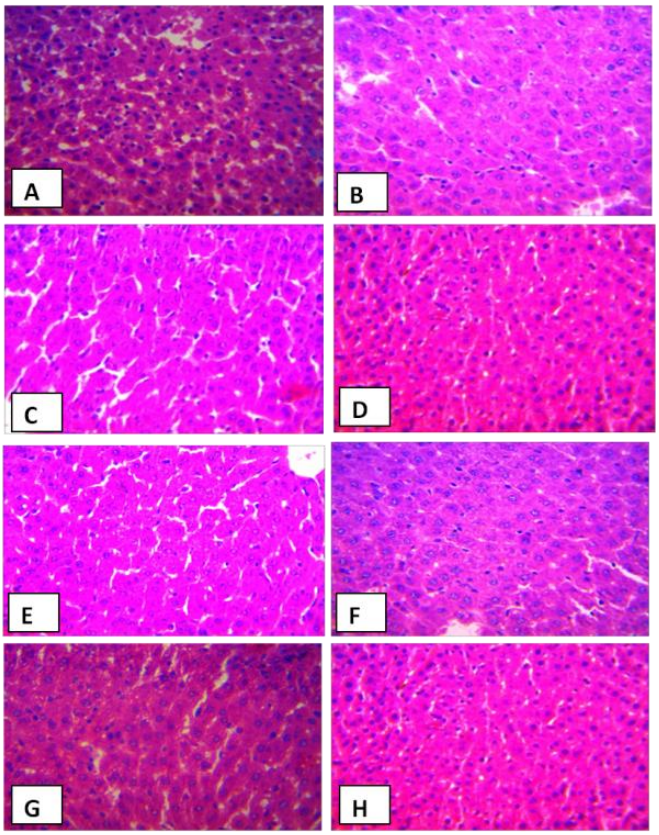

Plate 3
Liver histology (x400) of rats given aqueous fraction of Dacryodes edulis (De) leaf
A = rat given 10 mg/kg of De; B = rat given 100 mg/kg of De; C = rat given 1000 mg/kg of De; D = rat given 20 % Dimethyl sulphoxide (control I); E = rat given 1600 mg/kg of De; F = rat given 2900 mg/kg of De; G = rat given 5000 mg/kg of De; H = rat without treatment (control II). All show no visible lesions.

DISCUSSION

Dacryodes edulis leaf extracts revealed the richness of relevant phytochemical constituents. Its leaf has saponins, tannins, alkaloids, anthraquinones and flavonoids, thus is similar to Anyam *et al.* (2015) who reported the same phytochemical compounds for the hexane, chloroform, ethyl acetate and methanol extracts of boiled seed of *D. edulis* . In recent times, there is an increase in demand of saponins owing to their numerous biological, medicinal, and pharmaceutical applications. These include hemolytic factor, anti-inflammatory, antibacterial, antifungal, antiviral, insecticidal, anticancer, cytotoxicity, molluscicidal; and lowering of cholesterol in animals and man (El Aziz *et al* ., 2019). Alkaloids possess anti-amyloid, anti-inflammatory, antioxidant properties; anti-depressive and anti-convulsing efficacy (Ghulam *et al* ., 2018). Hence its high concentrations in both methanol extract and leaf powder of *D. edulis* is of great value.

In Ayurveda, tannin-rich plants are formulated for treating diseases like leucorrhoea, rhinnorhoea and diarrhea. Tannins are also applied in the dyeing, photography, brewery and wine industries as well as astringent in medicinal preparations (Belonwu *et al* ., 2014).

Furthermore, Okanlawon *et al* . (2015) expatiated that tannins prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them. Banso and Adeyemo (2007) added that since tannins have considerable toxicity against bacteria and fungi, they might assume pharmacological significance in future. Oyesomi and Ajao (2011) explained that when tannins are consumed, they reduce the permeability of the intestinal lining and thus reduce the amount of toxins from the microorganisms assimilated into the blood. This reduces the harmful effects the toxins may have, as well as the body's reaction to diarrhoea and dehydration (Aderiye *et al.,* 2015). Medicinal plants that are rich in tannins are used as antiseptic. In this study, ethyl acetate of *D. edulis* contained the highest tannins.

Hexane fraction had the best phenolic compounds, followed by butanol and ethyl acetate fractions. Many phenolic compounds possess antioxidant, antibacterial, viricide, anticancer, and anti-atherosclerosis (Elnour *et al* . 2018). Phenolics also exhibit anti-diabetic, anti-obesity, immunomodulatory, cardioprotective, hepatoprotective, and neuroprotective effects through antioxidant and anti-inflammatory activities (Priyanka *et al* ., 2019). Anthraquinones have diuretic, antimicrobial, anti-inflammatory and, anticancer properties (Chien *et al* ., 2015).

They are also useful as laxative, anti-tumor, antimicrobial, antiviral and antiparasitic (Fouillaud *et al* ., 2018).

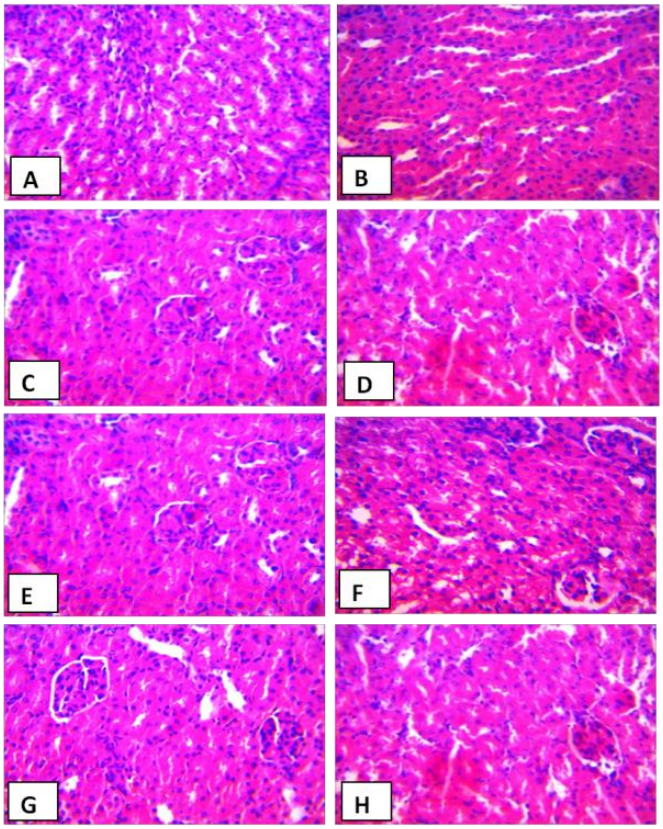

Plate 4:
Kidney histology (x400) of rats given aqueous fraction of *Dacryodes edulis* (De) leaf
A = rat given 10 mg/kg of De; B = rat given 100 mg/kg of De; C = rat given 1000 mg/kg of De; D = rat given 20 % Dimethyl sulphoxide (control I); E = rat given 1600 mg/kg of De; F = rat given 2900 mg/kg of De; G = rat given 5000 mg/kg of De; H = rat without treatment (control II). All show no visible lesions.

Flavonoids have anti-oxidative, anti-inflammatory, anti-mutagenic, anti-viral, anti-diabetic, anti-carcinogenic, anti-aging, and cardioprotective properties, and are nowadays regarded as a vital constituent in nutraceutical, pharmaceutical, medicinal and cosmetic applications (Panche *et al* ., 2016; Tian-yang *et al* ., 2018). The leaf of *D. edulis* is richest in flavonoids of powder leaf and butanol fraction, thus supports Tee *et al* . (2014) who reported that its fruits and leaves have flavonols.

Terpenes are useful in food, cosmetics, pharmaceutical and biotechnology industries (Thimmappa *et al* ., 2014). Moreso, there is renewed interest in both triterpenoid and steroidal saponins because of their wide chemical diversity (Yi *et al* ., 2019). In this study, butanol fraction of *D. edulis* leaf had the highest terpenoids, and same for carotenoids followed by aqueous and ethyl acetate fractions. Carotenoids are linked with antioxidant properties and some of them have activity of pro-vitamin A (Alan and Marina, 2014). The most significant use of the cardiac glycosides is its effects in treatment of cardiac failure (Nagy, 2017). Steroids are used in treating prostate cancer (Lubik *et al* ., 2016). The methanol extract, fractions and the pulverized leaf of *D. edulis* are very low in cardiac glycosides but fairly rich in steroids.

According to Abdullahi *et al* . (2008), high values of saponins, tannins, flavonoids and terpenoids in *D. edulis* leaf extracts have been reported to have antidiarrhoeal property in experimental animal models. Additionally, *D. edulis* leaf extracts demonstrated significant antimicrobial potency that might be linked to its high content in tannins as evident in its ethyl acetate fraction, thus supported the report of Ajibesin *et al* . (2011).

The methanol extract of *D. edulis* leaf was not active against *S. aureus*; contrarily, Okwu and Ighodaro (2009) reported activity of the oil from ethanol stem bark extract of *D. edulis* against *S. aureus.* This might be because of the different plant parts used. However, the ethyl acetate fraction of *D. edulis* leaf showed activities against *S. aureus, K. pneumoniae* and *P. aeruginosa*, thus supporting the report of Anyam *et al* . (2015) in which the ethyl acetate fraction of *D. edulis* seed was active against the same set of microbes.

This study has shown the safety of *D. edulis* . However, there was a mild lesion in the livers of rats dosed with 5000 mg/kg ethyl acetate fraction of *D. edulis* leaf.

In conclusion, the leaf of *Dacryodes edulis* was very rich in phytochemical constituents that supported some of the tradomedical claims. The solvent of extraction determined the phytochemical that could be optimally extracted. Ethyl acetate fraction was the most potent fraction against all the bacteria and three of the six molds experimented, thus might be a lead in formulating herbal drugs to combat diseases caused by such susceptible microorganisms. Ethyl acetate and aqueous fractions of *D. edulis* leaf were safe at 2900 and 5000 mg/kg doses, respectively.

ACKNOWLEDGEMENT
Dr. Oluwasanmi O. Aina (Veterinary Anatomy, University of Ibadan).

REFERENCES

Abdullahi A. L., Agho M. O., Amos S., Gamaniel K. S., Wambebe, C. (2008). Antidiarrhoeal activity of the aqueous extract of *Terminalia avicennoides* roots. Phytother Res 15: 431 - 434.
Aderiye B. I., David O. M., Atere, V. A. (2015). Administration of cashew extracts in the treatment of some infections and diseases. Advancement in Medicinal Plant Research 3 (3): 75 - 86.
Ajibesin K. K. (2011). Dacryodes edulis (G. Don) H. J. Lam: a review on its medicinal, phytochemical and economic properties. Research Journal of Medicinal Plant 5 (1): 32 - 41.
Ajibesin K. K, Bala D. N, Umoh U. F. (2011). The use of medicinal plants to treat sexually transmitted disease in Nigeria: Ethnomedical survey of Niger Delta region. Int J Green Pharm 3 (5): 181 - 191.
Alan G. O. S., Marina V. S. (2014). Main food sources of carotenoids, according to the purpose and degree of processing, for beneficiaries of the 'Bolsa Família' in Brazil. Food Sci. Technol, Campinas, 34 (2): 408 - 4 15.
Anyam J. N., Tor-Anyiin T. A., Igoli J. O. (2015). Studies on *Dacryodes edulis* II: phytochemical and medicinal principles of boiled seeds. International Journal of Current Research in Chemistry and Pharmaceutical Sciences 2 (4): 32 - 37.
Arekemase M. O., Oyeyiola G. P., Aliyu M. B. (2011). Antibacterial activity of *Anacaridum occidentale* on some enterotoxin producing bacteria. Int J Biol 3 (4): 968 - 973.

Banso A., Adeyemo S. O. (2007). Evaluation of antimicrobial properties of tannins isolated from *Dichrostachys cinerea*. African Journal of Biotechnology 6 (15): 1785 - 1787.

Belonwu D. C., Ibegbulem C. O., Nwokocha M. N., Chikezie, P. C. (2014). Some phytochemicals and hydrophilic vitamins of *Anacardium occidentale*. Research Journal of Phytochemistry 8: 78 - 91.

Burkill H. M. (2004). The useful plants of west tropical Africa. Ed. 2. Vol. 6. General index. Royal Botanic Gardens, Kew; 189 p.

Chien S., Wu Y., Chen Z., Yang W. (2015). Naturally Occurring Anthraquinones: Chemistry and Therapeutic Potential in Autoimmune Diabetes. Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine. Article ID 357357, 13 pp. http://dx.doi.org/10.1155/2015/357357.

El Aziz M. M. A., Ashour A. S., Melad A. S. G. (2019). A review on saponins from medicinal plants: chemistry, isolation, and determination. J Nanomed Res. 8 (1): 6 - 12.

Elnour A. A. M., Mirghani M. E. S., Musa K. H., Kabbashi N. A., Alam M. Z. (2018). Challenges of Extraction Techniques of Natural Antioxidants and Their Potential Applications Opportunities as Anti-Cancer Agents. Health Sci J 12 (5): 596.

Fouillaud M., Yanis C., Mekala V., Isabelle G., Laurent D. (2018). Anthraquinones. Leo M. L. Nollet; Jan*et al* ejandra Gutiérrez-Uribe. Phenolic Compounds in Food Characterization and Analysis, CRC Press, pp. 130 - 170.

Ghulam H., Azhar R., Haseeb A., Nimra A., Aroona R., Wei W., Muhammad A., Jiang L., Xiaomeng L. (2018). Role of Plant Derived Alkaloids and Their Mechanism in Neurodegenerative Disorders. Int. J. Biol. Sci. 14 (3): 341-357.

Harborne J. B. (1998). Phytochemical methods – a guide to modern techniques of plant analysis. Chapman and Hall: London.

Lorke D. (1983). A new approach to practical acute toxicity testing. Arch. Toxicol 54: 275 - 287.

Lubik A. A., Nouri M., Truong S., Ghaffari M., Adomat H. H., Corey E., Cox M. E., Li N., Guns E. S., Yenki P., Pham S., Buttyan R. (2016). "Paracrine Sonic Hedgehog Signaling Contributes Significantly to Acquired Steroidogenesis in the Prostate Tumor Microenvironment". Int. J. Cancer. 140 (2): 358 - 369. doi:10.1002/ijc.30450. PMID 27672740.

Marcano L., Hasenawa D. (1991). Analysis of phytochemicals in leaves and seeds. Agronomy Journal 83: 445 - 452.

Mayuri P. N. (2012). Screening of Ailanthus excelsa Roxb. for secondary metabolites. Journal of Current Pharmaceutical Research 10 (1): 19 - 219.

Nagy M. M. (2017). Aromatic and Medicinal Plants - Back to Nature. INTECH. pp 29 - 45. http://www.intechopen.com/books/aromatic-and-medicinal-plantsback-to-nature.

Okanlawon T. F., Ariwaodo J. O., Adeniji K. A. (2015). Phytochemical and antimicrobial properties of the genus Pericopsis thwaites (Papilionaceae) in Nigeria. International Journal of Scientific and Technology Research 4: 6.

Okwu D. E., Ighodaro B. U. (2009). GC-MS evaluation of the bioactive compounds and antibacterial activity of the oil fraction from the stem barks of *Dacryodes edulis* G. Don Lam Int J. Drug Dev. and Res 1 (1): 117.

Omonhinmin A. C. (2014). Ethnobotany of Dacryodes edulis (G. Don) H. J. Lam in Southern Nigeria 2: Practices and applications among the Igbo-speaking people. Ethnobotany Research and Applications 12: 071 - 080.

Orwa C., Mutua A., Kindt R., Jamnadass R., Anthony S. (2009). Agroforestree Database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya.

Oyesomi T. O., Ajao M. S. (2011). Histological effect of aqueous extract of *Anacardium occidentale* (cashew) stem bark on adult Wistar rat testis. Med Pract Rev 2 (7): 73 - 77.

Panche A. N., Diwan A. D., Chandra S. R. (2016). Flavonoids: an overview. Journal of Nutritional Science, 5 (47): 1 - 15. doi:10.1017/jns.2016.41.

Priyanka S., Anupam D. T., Rajat N., Satyajit D. S., Lutfun N., Jagajjit S., Manabendra D. C. (2019). Role of Natural Phenolics in Hepatoprotection: A Mechanistic Review and Analysis of Regulatory Network of Associated Genes. Front. Pharmacol., https://doi.org/10.3389/fphar.2019.00509.

Sidhu M. C., Sharma T. (2013). A database of antidiabetic plant species of family Asteraceae, Euphorbiaceae, Fabaceae, Lamiaceae and Moraceae. International Journal of Herbal Medicine 1 (2): 187 - 199.

Sodha R., Gaonkar S., Kolte S., Padmanabha, P. (2015). Antibacterial and antifungal activity of crude coconut shell oil. International Research Journal of Biological Sciences 4 (11): 16 - 20.

Tee L. H., Yang B., Nagendra K. P., Ramanan R. N., Sun J., Chan E., Tey B. T., Azlan A., Ismail A., Lau C. Y., Jiang Y. (2014). Nutritional compositions and bioactivities of Dacryodes species: a review. Food Chemistry 165: 247 - 255.

Thimmappa R., Geisler K., Louveau T., O'Maille P., Osbourn A. (2014). "Triterpene biosynthesis in plants". Annual Review of Plant Biology. 65: 225 - 257. doi:10.1146/annurev-arplant-050312-120229. PMID 24498976.

Tian-yang W., Qing L., Kai-shun B. (2018). Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. Asian Journal of Pharmaceutical Sciences 13 (1): 12 - 23.

Yi H., Zhuoyu H., Aoran L., Zhenzhou Z., Ning Y., Zixuan Y., Jingren H., Chengtao W., Sheng Y., Shuiyuan C. (2019). Recent Advances in Biotransformation of Saponins. Molecules. 24 (13): 2365. PMCID: PMC6650892.