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RESEARCH ARTICLE

Chemical characterization of Hymenocardia acida stem bark extract and modulation of selected enzymes in Kidney and Heart of Wistar rats

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ABSTRACT

Hymenocardia acida tul leaf and stem bark are used in treatment of several diseases in Africa. We examined the chemical constituents of the stem bark extract and its effects on some antioxidant indices and esterases in Wistar rats. Hymenocardia acida stem bark extract (HASBE) was obtained by Soxhlet extraction using methanol, followed by Atomic Absorption Spectroscopy (AAS), Fourier-Transform Infrared (FT-IR) spectroscopy, ultraviolet (UV) spectroscopy, High-performance liquid chromatography (HPLC) and Gas Chromatography-Flame ionization detection (GC-FID). Forty-eight male Wistar rats were assigned into eight groups (6 rats each), and administered orally with normal saline (Control), 50, 100, 150, 200, 250, 300, 350 mg/kg of HASBE twice per week for eight weeks. The rats were sacrificed under chloroform anesthesia, and kidneys and heart were excised, and processed into homogenates. Superoxide dismutase (SOD), catalase, lipid peroxidation (LPO), glutathione peroxidase (GPx), acetylcholinesterase (AChE) and carboxylesterases (CES) were determined spectrophotometrically. The AAS of HASBE detected Cobalt, Copper, Zinc, Iron, Nickel, Chromium, Manganese and Magnesium. The FT-IR shows four peaks as 2961.4, 2926.0, 1056.7 and 1034.3 cm-1, while UV shows absorbance between 250 nm and 650 nm. The HPLC identified orientin, β-sitosterol, rutin and betulinic acid, while GC-FID identified rutin, orientin, stigmasterol, hymenocardine and homopterocarpin as prominent compounds. The SOD significantly ($p < 0.05$) reduced in kidneys, while catalase was elevated in kidney and heart, with an increase in LPO level only in heart, relative to controls. The GPx, AChE and CE activities in kidneys were increased by HASBE, whereas, CE activity was lowered in heart. This study has demonstrated that Hymenocardia acida stem bark extract majorly contains iron, nickel, orientin, rutin, stigmasterol, hymenocardine, β-sitosterol, homopterocarpin and betulinic acid, and could possibly modulate the activities of antioxidant and esterase enzymes in kidney and heart of Wistar rats.

Keywords: Hymenocardia acida, elemental analysis, compound identification, enzyme activity.

Introducution

Hymenocardia acida tul is a plant commonly used in African traditional medicine[1,2]. The plant grows as a samll tree, which can be up to 6 – 10 m in height. *H. acida* has been reported to thrive on loamy, clayey and sandy soils in the Savanna and deciduous woodland areas[3]. The genus, hymenocardia has been described as a distinct faminly among all genera of Euphorbiaceae plants in Nigeria due to presence of winged-fruits[4]. The different tribes in Nigeria called the plant by several names, including Orupa (Yoruba), Yawasatoje (Fulfude), Enanche (Idoma), emela (Etulo), Ikalaga (Igbo), Ii-kwarto (Tiv) and Uchuo (Igede)[5,6].

Several people across the world have employed the use of botanical agents in treatment of different diseases[7], and these agents serve as good sources of developing modern drugs[6]. The name Hymenocardia is a Greek word 'hymen', meaning 'membrane', while 'kardia' means 'heart'[8]. A study by Udeozo *et al*[9]. revealed that H. acida contains chemical components, including cellulose, hemicelluloses and lignin. An earlier investigation by Sofidiya *et al*[10]. documented the use of Thin-layer chromatography to identify phenols, steroids, triterpenoids and flavonoids in the leaves of *H. acida.* Methanol leaf extract of *H. acida* inhibited the contraction of tracheal smooth muscle, while the analgesic potential of the root bark was reported by Olotu *et al*[11]. The potentials of *H. Acida* against certain pathogenic bacteria, such as *Staphylococcus auricularis*, *S. aureus*, *Streptococcus mutans Streptococcus pyrogens* and *Bacillus subtilis*, as well as and fungi (*Aspergilus flavus* and *Candida albinicans*) have been reported by Oshomoh and Ndu[12]. A study carried out by Adedokun *et al*[13]. documented that *Hymenocardia acida* possesses anticancer potential, which could be boosted via encapsulation in a polymeric material, Poly (lactic-co-glycolic acid) (PLGA). *H. acida* has shown potential to ameliorate neurodegenerative diseases[14].

The acute toxicity studies of methanol leaf and root bark extract of *H. acida* showed no mortality even at a dose as high as 2000 mg/kg[15]. Another investigation carried out by Koffi *et al*[16]. noted that in rodents, aqueous extract of *H. acida* roots administered at a sub-chronic level, via intravenous injection was non-toxic up to 1000 mg/kg, but could be harmful at higher doses. Sofidiya *et al*[10]. reported that the antibacterial and antioxidant potentials of *H. acida* leaves extract could be due to the phytochemicals, including phenols, flavonoids and triterpenoids, present in the extract.

The present study was designed to elucidate the chemical nature of methanol extract of *Hymenocardia acida* tree bark, and the possible modulatory roles it could play on the activities of some antioxidant and esterase enzymes in the renal and cardiac organs of male Wistar rats.

2.0 Materials and Methods

2.1 CHEMICAL AND REAGENTS

The study used chemicals obtained from the Sigma-Aldrich, Missouri, U. S. A. and British Drug House (BDH) Chemicals Limited, Poole, England. The chemicals have good analytical grades.

2.2 COLLECTION AND EXTRACTION OF *HYMENOCARDIA ACIDA* STEM BARK

Stem bark of *Hymenocardia acida* was collected in February, 2021, within the Campus of Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. After drying in the laboratory

at the room temperature for about three (3) weeks, the bark was blended using a mechanical grinder. The blended tree bark was subjected to methanol Soxhlet extraction, followed by rotary evaporation and oven-drying, to obtain dark brown powder labeled as *Hymenocardia acida* stem bark extract (HASBE).

2.3 ELEMENTAL DETERMINATION BY ATOMIC ABSORPTION SPECTROSCOPY

The HASBE was analyzed to determine the concentrations of Cobalt, Copper, Zinc, Iron, Nickel, Manganese, Magnesium and Chromium using Atomic Absorption Spectroscopy (AAS).

2.4 FOURIER-TRANSFORM INFRARED AND ULTRAVIOLET SPECTROSCOPY

The HASBE was analyzed using FT-IR (Agilent Cary 630 FTIR spectrophotometer). Wavelength was expressed in reciprocal centimeter (cm^{-1}). The UV analysis of HASBE was carried out using a UV- 1800 series spectrophotometer (Shimadzu) at the wavelength of 340 nm. Spectral data obtained were compared with literature data.

2.5. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ANALYSIS

The phytochemical profiling of HASBE was done using an isocratic HPLC machine (Mumbai, India) at a flow rate of 0.5 mL/min. Exactly 25 mg of the extract was dissolved in the mobile phase (acetonitrile and methanol- 80:20, v/v), and the injection volume was 20µL. The C18 (4.5 x 250 mm, 5µm) column was maintained at the room temperature and the eluent was detected at 210nm with a run time of 30 minutes. The peaks were compared with the inbuilt standard available in the NIST 11 library.

2.6 GAS CHROMATOGRAPHY-FLAME IONIZATION DETECTION ANALYSIS

The GC-FID identification of phytochemical compounds in HASBE was performed on HP SERIES II (5890) coupled to a flame ionization detector. Nitrogen was used as the carrier gas at the flow rate of 20 ml/min and hydrogen/compressed air was used as the combustion gas at the flow rate of 45 ml/min. The initial, injector and detector temperatures were 50^0C, 220^0C and 270^0C, respectively, while the oven was maintained at 240^0C at the rate of 10^0C/min, with a holding time of 2 minutes. Constituents were identified by comparing the mass spectra with the standard available in the NIST 11 library. The peak area of each constituent was used to estimate the percentage composition.

2.7 EXPERIMENTAL DESIGN

Forty male Wistar rats (average weight of 135.52 g), purchased from the Ladoke Akintola University of Technology Animal House, were randomly divided into eight groups (5 rats per group) and a then acclimatized for 7 days. Rats groups were treated as Control (Corn oil), 50, 100, 150, 200, 250, 300 and 350 mg/kg of HASBE (in corn oil) every other day, via oral gavage. After 28 days, rats were sacrificed using chloroform anesthesia, and kidney and heart tissues were excised and processed to obtain homogenates used for biochemical analyses.

2.8 BIOCHEMICAL ASSAYS

Total protein concentrations of kidney and heart homogenates were measured spectrophotometrically using the Biuret method of Lowry *et al*[17]. Activity of superoxide dismutase of tissues was estimated using the method of Misra and Fridovich[18]. Catalase activities of the renal and cardiac tissues were measured according to the method of Aebi[19], while lipid peroxidation level was determined by the method of Ohkawa *et al*[20]. The glutathione peroxidase activity was estimated by the method

of Paglia and Valentine[21]. Acetylcholinesterase activity in tissue homogenates was determined using the method described by Ellman[22], and Nachmansohn and Neumann[23], while Carboxylesterase enzyme activity was measured by the method described by Clement and Erhardt[24]. using paranitrophenyl acetate as substrate for the enzyme.

3.0 Results

The result in figure 1 shows the Atomic Absorption Spectroscopy of HASBE, revealing the presence of cobalt (0.303), copper (0.222), zinc (0.137), iron (2.027), nickel (1.304), manganese (0.213), magnesium (0.337) and chromium (0.313 ppm). The HASBE was analyzed using FT-IR spectroscopy, which showed prominent peaks at 2961.4 cm^{-1} (N-H stretching), 2926.0 cm^{-1} (carbonyl group), 1056.7 cm^{-1} and 1034.3 cm^{-1} (C-O stretching and aromatic rings) (Figure 2). The results in figure 3 revealed the UV spectroscopic analysis of *Hymenocardia acida* stem bark extract. Four prominent peaks were observed at wavelengths 250nm, 300nm, 390nm and 650nm. The HPLC Chromatogram of the HASBE reveals the presence of compounds with their retention times and percentage areas as, orientin (3.166 mins, 77.01%), β-sitosterol (4.061 mins, 6.58%), rutin (1.983 mins, 5.02%) and betulinic acid (1.650 mis, 3.33%), lupeol (9.730 mins, 1.80%), chromon (8.616 mins,3.33%), friedelin (8.183 mins, 0.73%), sigmasterol (7.530 mins, 1.64%),coumarin (6.350 mins, 2.20%) and 3-hydroxybenzoic acid (1.350 mins, 1.80%) as shown figure 4. As shown in figure 5, the GC-FID chromatogram of HASBE showed compounds (with their retention times and areas), including rutin (9.200 mins, 53.27%), orientin (5.016 mins, 13.06%) and stigmasterol (8.600 mins, 11.73%), hymenocardine (7.533 mins, 6.43%), homopterocarpin (7.81 mins, 5.29%), coumarin (6.033 mins, 0.40%), chromon (6.450 mins, 0.89%), paviin (7.150 mins, 2.41), 3-hydrobenzoicacd (3.300 mins, 3.22%), betulinic acid (4.033 mins, 1.57%), oleic acid (4.31 mins, 0.81%), sigmasterol (8.600 mins, 10.59%), friedelin (11.061 mins, 1.00%), squalene (11.483 mins, 2.67%), vitexin (11.910 mins, 0.32%), alphacolubrin (12.233 mins, 0.11 %), chelidonon (12.550 mins, 0.43%) and antathrone (14.566 mins, 0.36%)

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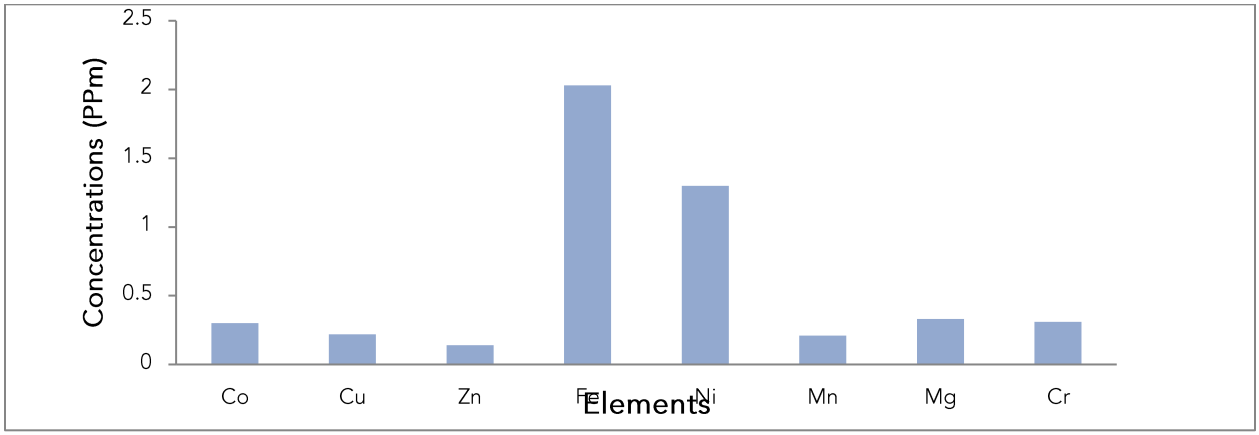

Fig. 1. Elemental profiling of *Hymenocardia acida* stem bark extract using Atomic Absorption Spectroscopy

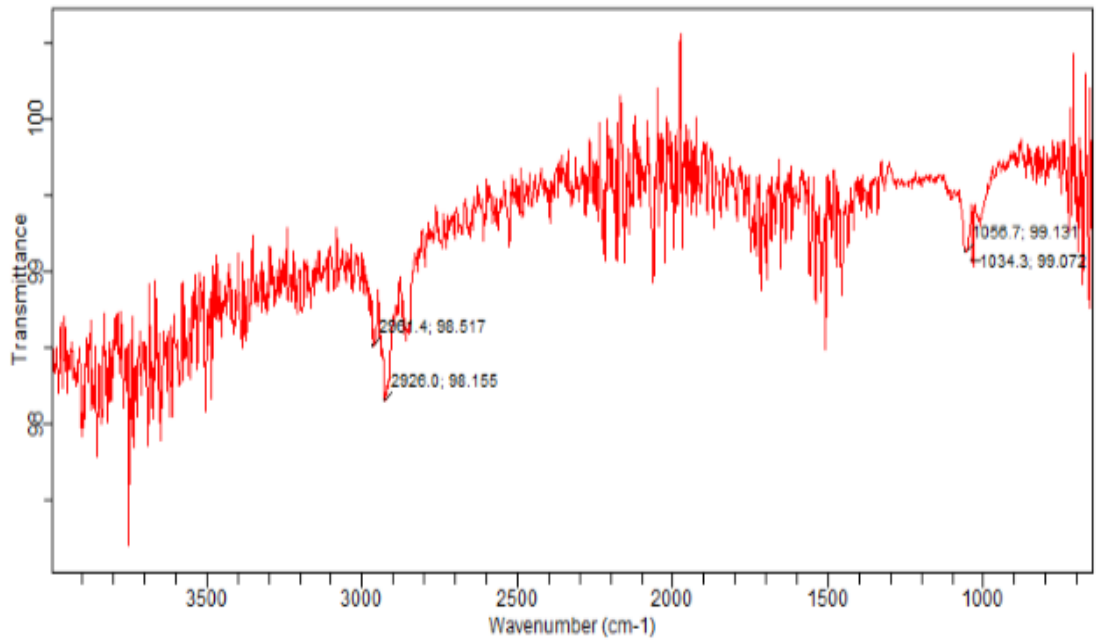

Fig. 2. Fourier Transform-Infra Red spectrum of Hymenocardia acida stem bark extract.

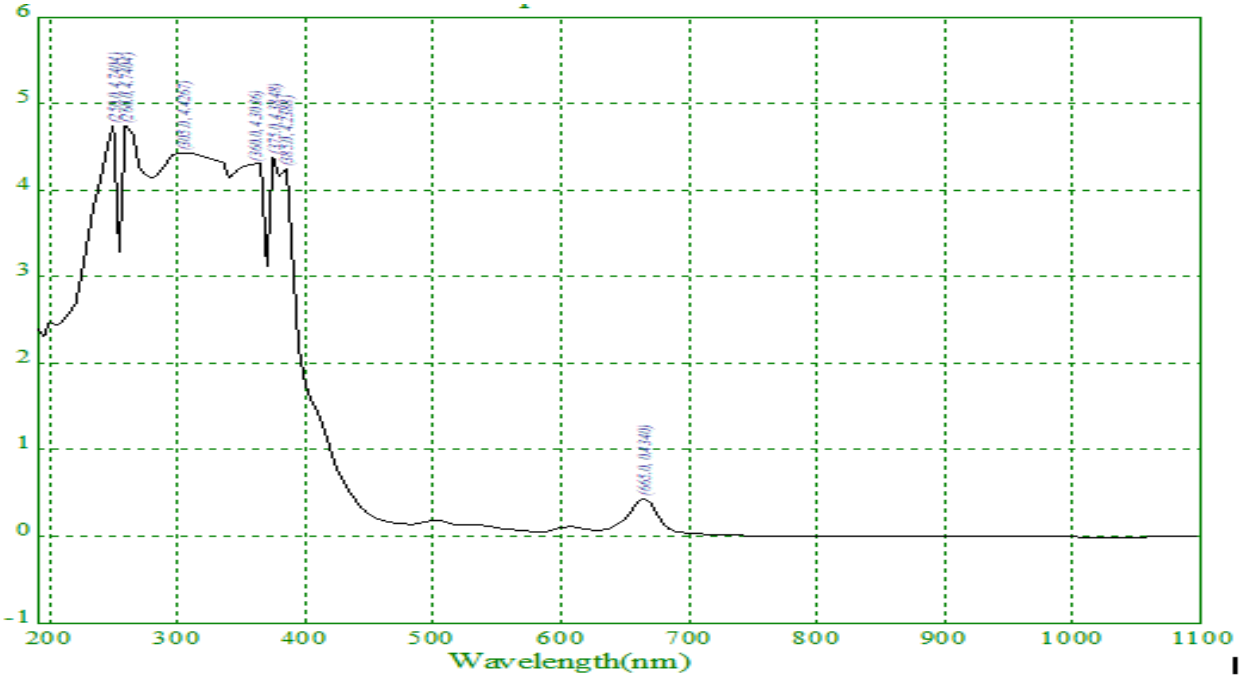

Figure 3. UV spectrum of Hymenocardia acida stem bark extract.

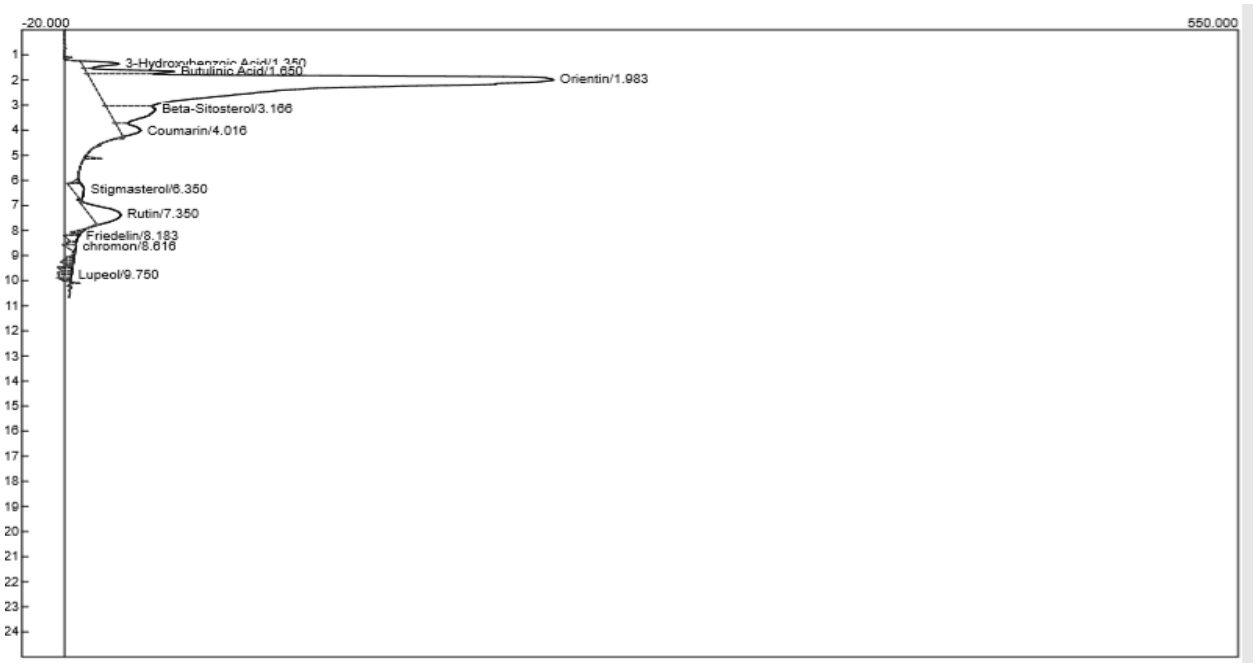

Fig. 4. HPLC Chromatogram of Hymenocardia acida stem bark extract

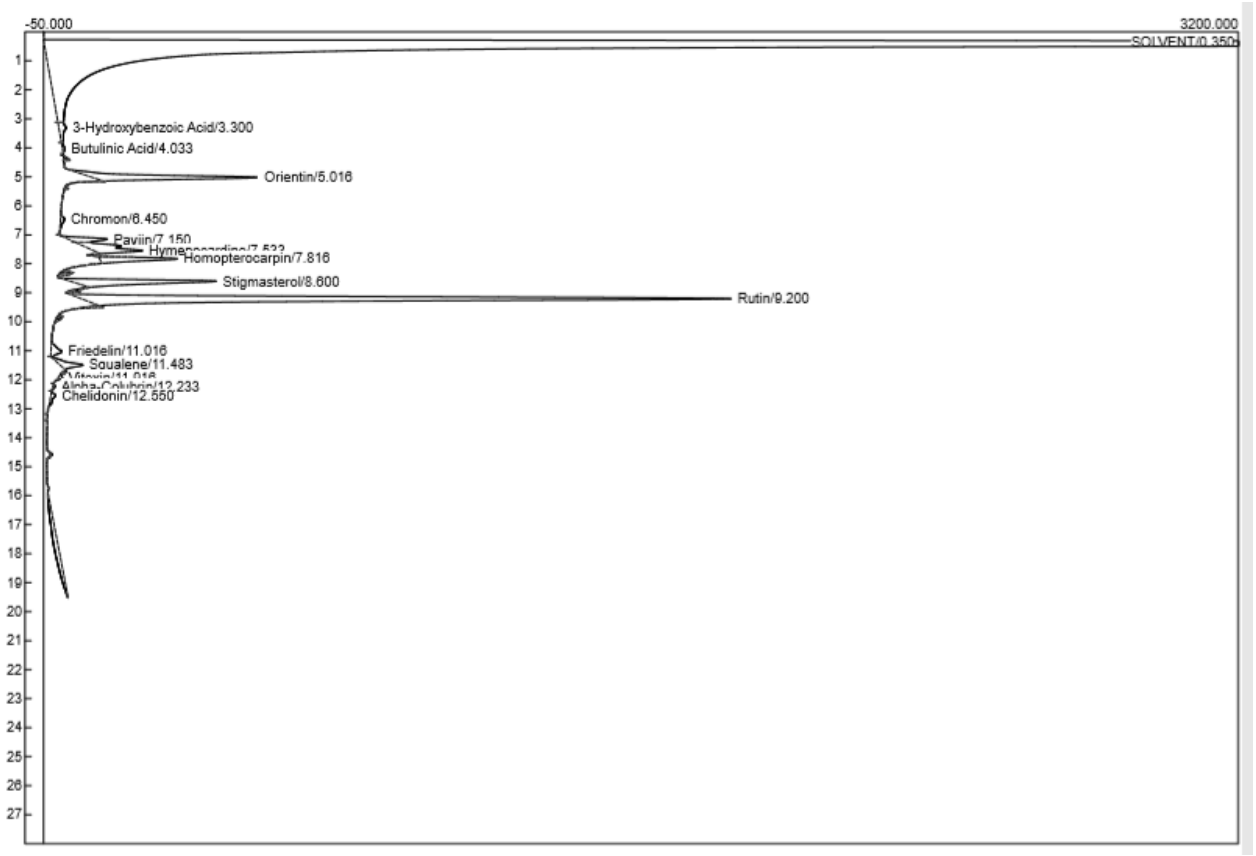

Figure 5. GC-FID Spectrum of Hymenocardia acida stem bark extract

In table 1, the activity of SOD was significantly ($p < 0.05$) reduced, while that of catalase was elevated, with no significant impact on lipid peroxidation level in kidneys of rats, across the treatment doses of 50 to 350 mg/kg, when compared to controls. However, the treatment

caused significant increase in both catalase and lipid peroxidation levels, with no significant effect on SOD in the cardiac tissue of the experimental rats, relative to control rats (Table 2).

The present study also investigated the effects of HASBE administration on the activities of glutathione peroxidase (GPx), acetylcholinesterase (AChE) and carboxylesterase (CE) enzymes in both renal and cardiac organs of the rats. Table 3 indicates that the activities of the three enzymes in the kidneys were increased across the 50 – 350 mg/kg doses used in the study, relative to control rats. However, table 4 shows that activity of AChE was significantly increased, while CE activity was lowered in the heart at 100 – 350 mg/kg doses, compared to controls. The activity of GPx was not significantly ($p > 0.05$) affected across all the treatment doses.

Table 1. Effects of *Hymenocardia acida* stem bark extract on SOD, Catalase and Lipid peroxidation in kidneys of rats

Treatments (mg/kg)	Superoxide dismutase activity x 10^{-5} (U/ mg protein)	Catalase activity (U/mg protein)	Lipid peroxidation (µM/mg protein)
Control	0.080 ±0.12	0.019 ± 0.01	1.484 ± 0.80
50	0.041 ±0.06#	0.020 ± 0.00	1.474 ± 0.11
100	0.043 ± 0.00#	0.036 ± 0.02	1.353 ± 0.10
150	0.044 ± 0.01#	0.025 ± 0.00	1.456 ± 0.15
200	0.043 ± 0.02#	0.020 ± 0.01	1.532 ± 0.04
250	0.046 ± 0.01#	0.027 ± 0.00	1.437 ± 0.11
300	0.056 ± 0.00#	0.020 ± 0.01	1.479 ± 0.06
350	0.039 ± 0.01#	0.022 ± 0.01	1.470 ± 0.06

Data expressed as Mean ± Standard deviation, N= 6

- Significantly lower compared to control, *- Significantly higher compared to control

Table 2. Effects of *Hymenocardia acida* stem bark extract on SOD, Catalase and Lipid peroxidation in heart of rats

Treatments (mg/kg)	Superoxide dismutase activity x 10^{-5} (U/mg protein)	Catalase activity (U/mg protein)	Lipid peroxidation (µM/mg protein)
Control	0.070 ± 0.01	0.037 ± 0.01	1.119 ± 0..09
50	0.074 ± 0.00	0.056 ± 0.00*	1.448 ± 0.08
100	0.077 ± 0.01	0.060 ± 0.02*	1.462 ± 0.16
150	0.075 ± 0.02	0.059 ± 0.00*	1.483 ± 0.15
200	0.076 ± 0.00	0.049 ± 0.02*	1.538 ± 0.11
250	0.075 ± 0.01	0.051 ± 0.00*	1.503 ± 0.09
300	0.072 ± 0.00	0.056 ± 0.01*	1.436 ± 0.10
350	0.079 ± 0.01	0.060 ± 0.03*	1.682 ± 0.15

Data expressed as Mean ± Standard deviation, N= 6

- Significantly lower compared to control, *- Significantly higher compared to control

Table 3. Effects of *Hymenocardia acida* stem bark extract on glutathione peroxidase, acetylcholinesterase and carboxylesterase activities in kidney of rats

Treatments (mg/kg)	Glutathione peroxidase activity (U/mg/protein)	Acetylcholinesterase activity (nmol/min/mg protein)	Carboxylesterase activity (nmol/min/mg protein)
Control	24.60 ± 4.00	2.50 ± 0.01	2.50 ± 0..06
50	52.00 ± 9.06*	6.35 ± 1.55*	5.00 ± 0.38*
100	49.25 ± 5.53*	5.28 ± 0.94*	6.65 ± 0.14*
150	83.20 ±10.90*	5.37 ± 0.03*	4.20 ±1.95*
200	72.39 ± 6.98*	3.68 ± 0.02*	6,53 ± 3.43*
250	71.50 ± 5.90*	5.90 ± 0.29*	7.64 ± 1.55*
300	65.54 ± 8.00*	6.26 ± 0.98*	4.67 ± 0.10*
350	81.89 ± 7.54*	5.87 ± 0.02*	8.25 ± 1.08*

Data expressed as Mean ± Standard deviation, N= 6

- Significantly lower compared to control, *- Significantly higher compared to control

Table 4. Effects of *Hymenocardia acida* stem bark extract on glutathione peroxidase, acetylcholinesterase and carboxylesterase activities in heart of rats

Treatments (mg/kg)	Glutathione peroxidase activity (U/mg/protein)	Acetylcholinesterase activity (nmol/min/mg protein)	Carboxylesterase activity (nmol/min/mg protein)
Control	1.81 ± 0.15	1.30 ± 0.01	7.03 ± 1.09
50	1.83 ± 0.08	1.20 ± 0.10	6.72± 1.20
100	1.75 ± 0.05	3.14 ± 0.07*	4.29 ± 0.96#
150	1.93 ± 0.03	2.35 ± 0.29*	2.45 ± 0.15#
200	1.77 ± 0.10	2.75 ± 0.12*	4.56 ± 1.00#
250	1.80 ± 0.15	4.89 ± 0.67*	3.67 ± 0.89#
300	1.73 ± 0.10	5.05 ± 0.01*	3.56 ± 0.10#
350	1.90 ± 0.07	4.22 ± 0.43*	4.87 ± 1.99#

Data expressed as Mean ± Standard deviation, N= 6

- Significantly lower compared to control, *- Significantly higher compared to control

Discussion

Several studies have indicated the importance of both the leaf and stem bark of *Hymenocardia acida tul* in Trado-medicine in the African region[1,2]. The reported Trado-medical potential of this plant could be due to presence of various secondary metabolic products, such as glycosides, flavonoids, alkaloids, tannins, saponins and terpenoids[6]. In this study, we chemically elucidated the methanol extract of *Hymenocardia acida* tree bark, and examined the responses of some antioxidant indices and two esterases in kidneys and heart of Wistar rats administered with varying doses of the extract.

Mineral elements are required by living organisms for normal physiologic processes. Investugation of the elemental composition of HASBE using the AAS technique showed the

presence of cobalt, copper, zinc, iron, nickel, manganese, magnesium and chromium. Among the eight elements determined in this study, iron and nickel were present in high concentration, while zinc was the least. Udeozo *et al*[9]. reported the presence of sodium, potassium, lead, calcium, zinc, magnesium, and copper in methanol extract of *H. acida*. In humans, iron is a component of heme and iron sulphur clusters.[25] Accumulation of iron has been linked to cardiomyopathy, involving oxidative injury, cardiac electrical interference and fibrotic development[26,27]. Since heart is a high-energy demanding organ, deficiency iron deficiency has been noticed in majority of patients with severe heart failure[28], and iron supplementation has been found to improve this condition, as well as exercise tolerance[29,30]. In this study, nickel was the second highest element determined. A study carried out by Stangl and Kirchgessner[31] revealed that nickel deficiency is associated with increased levels of serum triacylglycerols, cholesterol, low-density lipoprotein and iron deficiency. The presence of large amounts of iron and nickel, in stem bark of *H. acida* in this study, thus indicates that the extract could protect against cardiac failure and imbalance in lipid metabolism.

A versatile non-invasive technique used in detection of functional groups present in compounds is FT-IR spectroscopy. In the present study, the spectrum of HASBE shows four prominent peaks at 2961.4 cm^{-1} (indicating N-H stretching), 2926.0 cm^{-1} (indicating carbonyl/ ketone group), 1056.7 cm^{-1} and 1034.3 cm^{-1} (indicating C-O stretching of alcohols and aromatic ether groups, respectively)[32,33], as shown in figure 2. However, Adedokun *et al*[13]. reported that FT-IR spectrum of *H. acida* stem bark extract showed transmittance bands at 1600 cm^{-1}, 3100 to 3600 cm^{-1}. According to Shriner *et al*[32], these spectral bands indicate the presence of aromatic C=C bond and N-H (stretching) bond of heteroaromatics. Functional groups such as methyl stretching, carbonyl and aromatic groups have also been identified in an extract of *H. acida* stem bark[9]. The functional groups such as amine, ether, ketone and heteroaromatics being revealed from the FT-IR sp ectral data in this study are due to presence of orientin, rutin, hymenocardine and others in the extract. The UV spectral datum of *Hymenocardia acida* stem bark extract (Figure 3) has revealed four prominent peaks with absorptions at wavelengths ranging from 250nm to 650nm.

The HPLC Chromatogram of the HASBE (Figure 4) presents four major compounds, namely orientin, β-sitosterol, rutin and betulinic acid, while the GC-FID chromatogram (Figure 5) presents five major compounds, including rutin, orientin, stigmasterol, hymenocardine and homopterocarpin. The HPLC and GC-FID chromatograms in this study have presented orientin and rutin, respectively, as prominent compounds present in *H. acida*. It is noteworthy to state that the presence of the other compounds in relatively minute quantities does not suggest that they have no implications in pharmacological casework. Orientin is a water-soluble compound, and its IUPAC name is 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-4-one. Orientin, a flavonoid C-glycoside of luteolin, has been identified in the extracts of bamboo (*Phyllostachys nigra*)[34], pigeon pea (*Cajanus cajan*)[35] and *Vaccinium bracteatum*[36] leaves. This flavonoid has also been successfully isolated from dried flowers of *Trollius chinensis*[37] and

Trollius ledebouri[38] by means of HPLC technique. An HPLC analysis reported by Sar *et al*[39]. showed homoorientin, rutin and orientin as major compounds in *H. acida*. Lam *et al*[40]. documented that orientin possesses antiinociceptive, antibacterial, cardioprotective, neuroprotective, antiinflammatory and antidepressant properties. A study carried out by Kalaiyarasu *et al*[41]. depicted orientin as being capable of preventing cancer of the colon, induced by 1,2-dimethylhydrazine in albino rat model.

The stem bark of *H. acida* has been well documented to contain carbohydrates, saponins, tannins, alkaloids, flavonoids, terpenes and steroids[11,15] Rutin, with the IUPAC name 3, 3′ 4′ 5, 7-pentahydroxyflavone-3-rhamnoglucoside, is a biophenolic flavonoid glycoside with a myriad of therapeutic potentials[42]. Studies carried out by Park *et al*[43]. and Yu *et al*[44]. indicated the protective role of rutin against oxidative damage. Apart from flavonoids, H. acida has also been documented for presence of certain alkaloids, with a cyclopeptide nucleus, namely hymenocardine, hymenocardinol, hymenocardine N-oxide and hymenocardine-H[2] Hynenocardinol is a reductive metabolic product of hymenocardine, and the two compounds are identifiable in the urine and plasma of rats administered with hymenocardine[45]. Homopterocarpin, another molecule identified in the present study, is a flavonoid reported to possess both antioxidant and hepatoprotective properties[46]. Some other studies have also identified β-sitosterol, betulinic acid, stigmasterol, hymenocardine and homoorientin as phytochemicals in *H.acida*[47,48,49]. Adeleke and Adaramoye[50,51] reported the potential of betulinic acid shows protection against oxidative damage in kidney, liver and testes of rats.

The effects of HASBE administration on some antioxidant indices in kidneys and heart of experimental rats were also examined in this study. Table 1 shows that the activity of SOD was significantly reduced, while that of catalase was elevated in kidneys. The lipid peroxidation level was not however affected, when compared to controls. In the cardiac tissue, catalase and lipid peroxidation levels were elevated, whereas SOD activity was not affected, relative to control rats (Table 2). The findings thus suggest the potential of the extract to protect against oxidative injury in the two organs being examined. Oxidative stress results from an imbalance in the relative levels of antioxidant and pro-oxidant molecules, resulting in excess amount of reactive oxygen species (ROS) and compromised biological detoxification system[52]. At high levels, these molecules undergo oxidative reactions with biomolecules, leading to tissue injury and cell death[53,54,55]. Dismutation of superoxide ions to hydrogen peroxide is catalyzed by superoxide dismutase, while the peroxide is finally detoxified to water and oxygen by catalase. As reported by certain studies, the antioxidant activity of rutin is underlined by mechanisms involving its chemical structure as a direct scavenger of reactive oxygen species[56], increased production of GSH, up-regulation of antioxidant enzymes genes expression[57] and inhibition of xanthine oxidase, involved in catalyzing ROS formation[58].

The present study also investigated the effects of HASBE administration on the activities of glutathione peroxidase (GPx), acetylcholinesterase (AChE) and carboxylesterase (CE) enzymes in both renal and cardiac organs of the experimental rats (Tables 3 and 4). The activities of the three enzymes in kidneys were increased

at all the doses of HASBE used in the study. Detoxification of hydrogen peroxide through reduction of glutathione is catalyzed by glutathione peroxidase enzyme[59,60]. The elevation in catalase and GPx activities of renal organ, noticed in this study, showed the potential of HASBE to protect this organ from damage due to superoxide radical and hydrogen peroxide. When GPx activity in kidney is reduced, ROS generation increases resulting in inflammation, mitogenesis, and development of fibrosis in this organ[61].

This study has revealed elevation in activity of AChE in both heart and kidney, whereas the CE activity was increased in kidney, and lowered in heart of the experimental rats. Acetylcholinesterase is a major enzyme, catalyzing acetylcholine hydrolysis and synaptic transmission in the cholinergic system. The observation of AChE activity in kidney of the experimental rats in this study shows that this enzyme is also expressed in non-cholinergic tissues of the body. This is in agreement with a documentation by Soreq and Seidman[62], Revuelta *et al*[63], Lu *et al*[64]. and Mondal *et al*[65], which indicated presence of AChE in non-neuronal and non-cholinergic cells, being involved in growth regulation, cell adhesion and cell differentiation. Rutin, a prominent component detected in HASBE in this study, could inhibit acetylcholinesterase, making this flavonoid potent in the treatment of neurodegeneration caused by oxidative imbalance[66].

Carboxylesterases (CES) from mammalian sources belong to the superfamily of serine hydrolases. These enzymes are responsible for catalyzing cleavage of ester, amide ester and thioester bonds, not only in endogenous, but also environmental and therapeutic compounds, converting them to their corresponding carboxylic acids and alcohols[67,68,69]. Most studies have documented mammalian CES 1 and CES 2 families as the two major isoforms of the superfamily, and they have different tissue distribution, and specificities for substrate and inhibitors expressed in body tissues. The CES 1 isoform is majorly expressed in liver, and less in heart, kidney, testis and intestine[70], whereas CES 2 isoform is mostly expressed in colon and small intestine[71]. An *in-vivo* inhibition of CES 1 enzyme resulted in bioavailability and treatment efficacy of certain ester drugs due to their reduced metabolic clearance[72]. In the present study, activity of cardiac CES of the rats was reduced, while that of kidney was elevated. It could be suggested from this finding that HASBE has acted as an inhibitor of the cardiac CES, thus possibly increasing the bioavailability and efficacies of the phytochemicals it the extract.

This study has shown that *Hymenocardia acida* stem bark is rich in minerals and phytochemicals, which may be significant in development of drugs with possible protection of kidney and heart of Wistar rats, via pathways involving antioxidant and esterase enzymes.

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