Topic: GCMS Phytochemical Profiling and evaluation of the effect of methanol extract of *Anacardium occidentale* (cashew) stem bark on antioxidant and liver function markers of hepatotoxic rats

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Introduction

In the past few years, there has been a sudden increase in the hunt or search for natural source for antioxidants. This is as a result of the increasing evidence that implicates oxidants in the etiology of many diseases such as diseases of the liver, inflammatory diseases and neurological diseases. Simultaneously, there is an upsurge in the side effects of orthodox drugs used in the past few decades as the therapeutics for managing and treating these diseases.

Medicinal plants with minimal side effect have gained much attention as an alternative medicine useful for treatment, managing and prevention health defects or diseases. They are used to treat a wide variety of diseases and are potential natural hepatoprotectives and antioxidant compounds in the treatment of liver diseases. Most of the therapeutic effects of these plants has been attributed to their phytochemical constituent. *Anacardium occidentale* (Cashew) is one of such plants whose stem bark extract is used traditionally for the treatment of many diseases.

Aim

This study was aimed at phytochemical profiling of methanol extract of *Anarcardium occidentale* (cashew) stem bark (MEAOSB) and evaluation of its effect on antioxidant and liver function markers

Methods

Materials

Collection and Authentication of Plant Material

Fresh *Anarcardium occidentale* stem barks were collected from Ezimo in Udenu Local Government Area of Enugu State, Nigeria. The stem barks were identified and authenticated by a botanist in the Department of Plant Science and Biotechnology, University of Nigeria, Enugu State. The stem barks were air-dried under a shed and pulverized.

Management of Experimental Animals

Albino Wistar Rats of average body weight of 180-220 g were used for this study. The animals were purchased from the Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were acclimatized under standard laboratory condition in the animal farm of the Department of Biochemistry for one week prior to the commencement of the

experiment with a 12 hour light and dark cycle and maintained on a regular feed (commercial chicken grower's mash) and water *ad labium*. They received human care throughout the experimental period in accordance with the ethical rules and recommendations of the University of Nigeria committee on the care and use of laboratory animals and the revised National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No.85-23, revised 1985) at the Animal house, Department of Biochemistry.

Extraction Procedure

The fresh *Anacardium occidentale* stem bark were collected and washed to remove impurities. The plant material was shade-dried with regular turning to avoid decaying, until crispy. The dried leaves were pulverized into powdered form using a mechanical grinder. A known weight of the pulverized stem bark 500 grams was macerated in 3.2 L absolute methanol using a maceration flask. The mixture was left for 48 hours and tightly corked in a conical flask, after which it was filtered into a flat bottomed flask using a muslin cloth. Further filtration was achieved with Whatman No 1 filter paper so as to remove fine residues. The filtrate was concentrated using a rotary evaporator at 45℃ to obtain the crude methanol extract. The concentrated extract was stored in a labeled sterile screw-capped bottle at 2-8℃.

Experimental Design

In the experimental design of this study, a total of 25 Wistar albino rats divided into five groups, of five rats each, were used. Hepatotoxicity was induced with 2 ml/kg of carbon tetrachloride (CCl_4) in all the groups except group one (1) which served as normal control. Group 2 was induced but not treated and served as the positive control while group 3-5 were induced and treated with standard (silymarin), 200 and 400 mg/kg of the extract respectively. Treatment lasted a week. Phytochemical profiling was done with GCMS while biochemical analyses of the parameters were determined by standard protocol.

Biochemical parameters Assayed

The biochemical parameters analysed include alkaline phosphatase (ALP), aspartate transaminase (ASP), alanine transaminase (ALT) and total bilirubin, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), glutathione (GSH) reductase, glutathione transferase, glutathione and malondialdehyde. The method of Reitman and Frankel (1957) was used in determining the activity of the following enzymes ALT, ASP, ALP and GSHPx while MDA, CAT, GSH and SOD were respectively determined by Farombi *et a*l. (2002), Sinha (1972), Jollow et al. (1972) and Fridovic (1989).

Determination 0f Compounds Present Using GC-MS

Extraction of Phytochemicals

Ethanol (15 ml) was added to a test tube containing 1g of the extract. The test tube was allowed to react in a water bath at 60 ^{0}C for 60 minutes. After the reaction time, the reaction product contained in the test tube was transferred to a separator funnel. The tube was washed

successfully with 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water and 3 ml of hexane, which were all transferred to the funnel. This extract were combined and washed three times with 10 ml of 10 %v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000 µl of hexane of which 200 µl was transferred to a vial for analysis.

The GC–MS analysis of bioactive compounds from the extracts of the leaves was done using Agilent Technologies GC systems with GC- M910 model (Buck Scientific, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 –150 °C with increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the prepared 1% of the extracts diluted with respective solvents was injected in an splitless mode. Relative quantity of the volatile compounds present in the extract was expressed as percentage based on peak area produced in the chromatogram.

Bioactive compounds extracted were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC–MS systems).

Statistical Analysis

The data obtained were analyzed using both one way analysis of variance (ANOVA) in Statistical product and Service Solution (SPSS) version 20.0 and presented as Mean ±SD. Mean values with $p < 0.05$ of the result was accepted significant.

Results

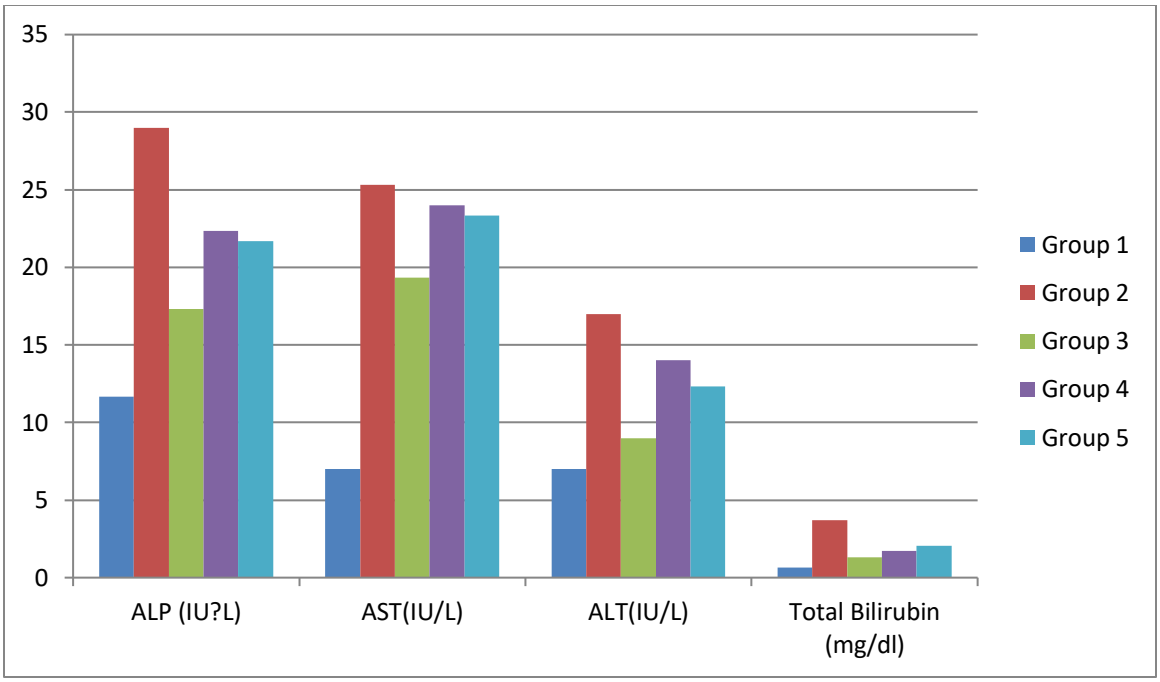

Figure 1: **Effect of methanol extract of cashew stem bark (MEAOSB) on liver markers in CCl_4-Induced hepatotoxic rats**

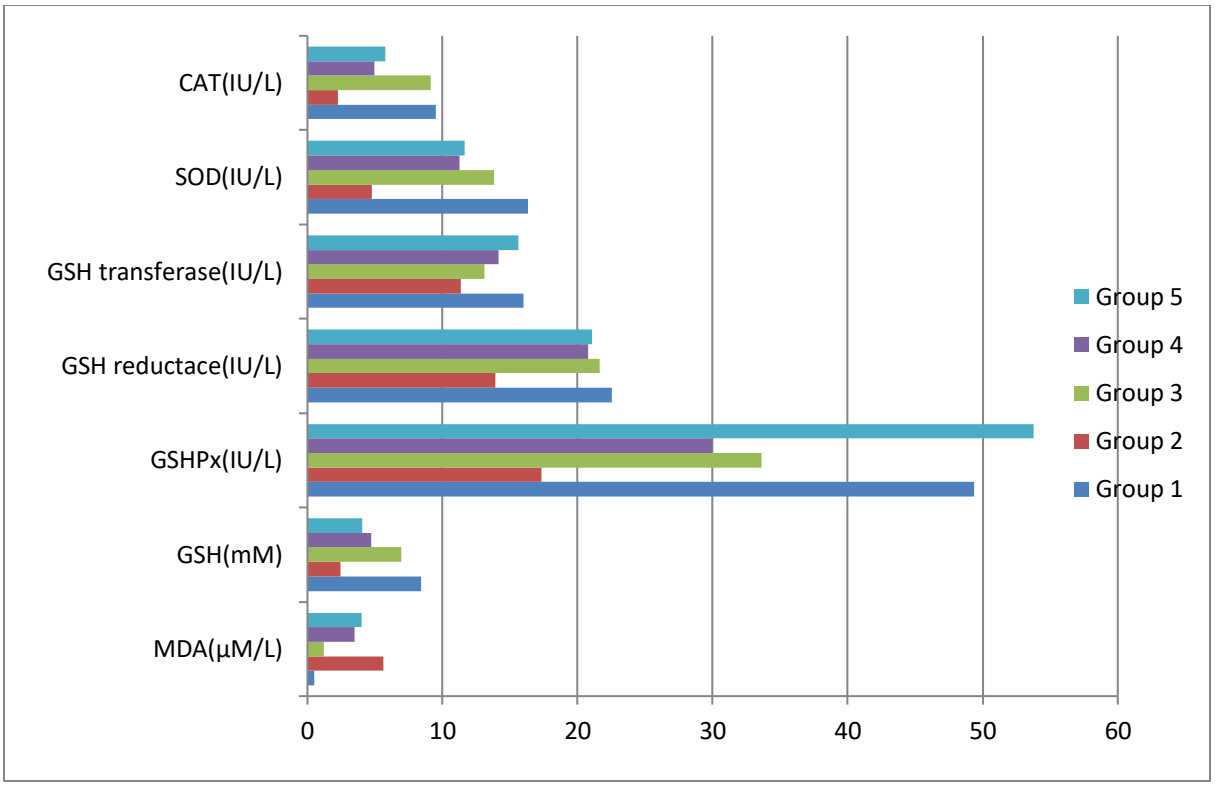

Figure 2: **Effect of methanol extract of cashew stem bark (MEAOSB) on antioxidant markers in CCl_4-Induced hepatotoxic rats**

Table 1: **GCMS phytochemical profile of MEAOSB**

Name of compound	Molecular weight (g/mol)	Molecular formula	Retention Time	Area %
Boric acid	61.83	H_3BO_3	2.341	2.40
Trimethyl ester	270.46	$C_{19}H_{36}O$	2.341	2.40
1-(trimethylsilyl) Oxy)propane 2-ol	148.28	$C_6H_{16}O_2Si$	2.341	2.40
Mercaptopropa-noic acid	106.14	$C_3H_6O_2S$	3.327	1.85
1-propane	58.08	C_3H_6O	3.327	1.85
3-methylthio methylsulfonyl 1phenyldithreitol	122.12	$C_4H_{10}O_4$	3.327	1.85
Methanamine	31.05	$C_8H_{15}N$	3.637	16.53
Cyanogen Chloride	61.470	CClN	3.637	16.53
Silane	32.12	SiH_4	4.116	3.45
Trimethyl silly ethane	91.135	$C_5H_{12}O_3Si$	4.116	3.45

peroxoate				
Hydrazine carbothioamide	91.135	CH_5N_3S	4.116	3.45
Cyclotrisiloxane	138.30	$H_6O_3Si_3$	4.538	2.58
Cyclopentane 1 carboxylic acid	112.12	$C_6H_8O_2$	4.538	2.58
Methyl Valerate	116.16	$C_6H_{12}O_2$	4.933	1.27
3 cyclopentane 1 acetaldehyde	152.23	$C_{10}H_{16}O$	5.234	0.29
2,5 dimethyl furan	96.13	C_6H_8O	5.243	0.29
3 amino 5-(indolyl)-4 pyrazolecarbonitrile	223.24	$C_{12}H_9N_5$	5.553	0.14
Indeno(2,1)pyridin 9-one	181.194	$C_{12}H_7NO$	5.553	0.14
Hexanoic acid	116.16	$C_6H_{12}O_2$	5.806	0.54
Octamethyl cyclotrisiloxane	296.62	$C_8H_{24}O_4Si_4$	6.116	2.51
Decane	142.29	$C_{10}H_{22}$	6.370	0.48
Tetradecane	198.39	$C_{14}H_{30}$	6.370	0.48
2,6,10 trimethyl decane	184.22	$C_{13}H_{38}$	6.370	0.48
Heptanoic acid	130.18	$C_7H_{14}O_2$	6.679	1.10
Undecane	156.31	$C_{11}H_{24}$	7.243	0.37
Dodecane	170.33	$C_{12}H_{26}$	7.243	0.37
Decamethyl Benzeneethanamine N-(Pentafluorophenyl) methylene)-beta 4 bis (trimethyl silly) Oxy)	475.60	$C_{21}H_{26}F_5NO_2Si_2$	7.412	6.03
1 fluoro dodecane	160.27	$C_{10}H_{25}$	8.060	1.05
1-octanol	130.23	$C_8H_{18}O$	8.060	1.05
Nonanoic acid	158.24	$C_9H_{18}O_2$	8.454	0.88
Dodecamethyl-cyclohexasiloxane	444.92	$C_{12}H_{36}O_6Si_6$	8.989	3.49
Aspermidin-17-ol	414.5	$C_{23}H_{30}N_2O_5$	14.990	0.21
2 Aziridinone	247.38	$C_{16}H_{25}NO$	15.694	1.63
3 Trimethyl silyl-oxy stearic acid	356.70	$C_{21}H_{44}O_2Si$	,16.257	0.71
Undecanoic acid	186.29	$C_{11}H_{22}O_2$	13.468	0.07
9 Tricosene	322.6	$C_{23}H_{46}$	13.947	0.01
Cis-vaccenic acid	282.46	$C_{18}H_{34}O_2$	14.060	0.01
1 pentadecene	210.40	$C_{15}H_3O$	14.483	0.15
Cycloocta siloxane	352.68	O_8Si_8	14.764	0.38

3 amino 2 phenazinol	211.22	$C_{12}H_9N_{30}$	14.764	0.38
11 oxo 9 Undecanoate	214.30	$C_{12}H_{22}O$	14.990	0.21
Decanoic acid	172.26	$C_{10}H_{20}O_2$	9.581	0.52
Pentanoic acid	102.13	$C_5H_{10}O_2$	9.581	0.52
Oxalic acid	90	$H_2C_2O_4$	10.539	0.52
Propyl undecyl ester	362.5	$C_{22}H_{34}O_4$	10.539	0.52
2 chloro propionic acid	108.52	$C_3H_5ClO_2$	12.820	0.03
Cyclohepta siloxane	519.08	$C_{14}H_{42}O_7Si_7$	11.750	1.09
Hexadecanoic acid	256.42	$C_{16}H_{32}O$	11.243	0.21
Hexadecane	226.41	$C_{26}H_{34}$	11.243	0.21
1 Octadecane sulphonyl chloride	353.0	$C_{18}H_{37}ClO_2S$	10.539	0.52

Discussion

As depicted in fig. 1 above, induction with CCl_4 was injurious to the hepatocytes as indicated by the leakage of ASP, ALT and ALP into the peripheral blood. This leakage may be due to generation of free radical, CCl_3• that alkylates cellular proteins and lipids in the presence of oxygen causing lipid peroxidation and consequently causing liver damage. Findings from this research indicate that high doses of CCl_4-induction could result in Oxidative and hepatic damage and MEAOSB has the potential of scavenging radicals and protecting the liver against CCl_4-induced oxidation and hepatotoxicity. The observed effect of MEAOSB could be due to the presence of Aspidospermidin-17-ol, hexadecanoic acid,methyl ester and Aziridinone in the extract shown by the GCMS analysis.

Conclusion

Findings from this research indicate that high doses of CCl_4-induction could result in hepatic damage and methanol extract of *Anacardium occidentale*stem bark has the potential of protecting the liver against CCl_4-induced hepatotoxicity. This hepatoprotective activity could be of great therapeutic potentials to clinicians, and is attributed to the antioxidant activities. It is recommended that the methanol stem extract of *Anacardium occidentale* should be used in the management of liver disorders.

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