

Phytochemical Comparative Screening of Aqueous Extracts of the Leaves, Stem barks, and Roots of *Hura crepitans* (L) using GC – FID.

Adindu, Eze Azubuike^{1*}, Elekwa, Iheanyichukwu¹ and Ogwo Joseph Ikedi²

Department of Biochemistry, Abia State University, Uturu, Abia State, Nigeria.

X-Trim Research Ltd, Aba, Abia State

Abstract: The phytochemical screening of the aqueous extracts of leaves, stem bark and roots of *Hura crepitans* were analysed using the Gas Chromatography – Flame Ionization Detector (GC – FID) method. The various parts of the plants were collected and air-dried under shade for one month after which they were sent for analysis. All the various parts showed varying amounts of phytochemicals: Flavonoids, Carotenoids, Sterols, Terpenes, Alkaloids, Saponins and Phenolic acids. The Aqueous Root Extracts (ARE) showed the highest amounts of the following phytochemicals when compared with other parts: phenolic acid content of 193.776mg/100g; flavonoids total content of 151.6604g/100g; alkaloid total content of 199.41g/100g; saponin total content of 130.005mg/100g; sterol total content of 4.02985g/100g and terpenes total content of 98.2341%. The leaves showed the highest carotenoid total content of 90.9181g/100g. The rich phytochemical content of these aqueous extracts especially of the roots suggests its potential use as a medicinal plant and may also have other industrial applications as raw materials.

Key words: *Hura crepitans*, Phytochemicals, Gas Chromatography – Flame Ionization Detector, Leaf, Bark, Roots.

I. Introduction

Hura crepitans (L) belongs to the family Euphorbiaceae. It is also known as the sandbox tree, possum wood or Jabillo (Duke, 1985; Morretti and Grenand, 1982). In the wild, the tree grows above 40m and the stem and branches are mainly spiny. In ancient times, the plant's bark extract was used to treat leprosy, eczema and in warfare (Barbieri, *et al*, 1983). Recently, a wide range of therapeutic applications have been found of the leaves, stem bark, roots and seeds of *Hura crepitans*. Such uses include: a strong purgative; astringent and emollient actions; dermatological uses; fungicidal, antimicrobial, etc (Poswal and Akpa, 1991; David, *et al*, 2014; Adedire and Ajayi, 2003).

Phytochemicals or secondary metabolites found in plant foods and other plant extracts are numerous and exhibit various physiological actions. Apart from the nutritive values of different plants foods and extracts, they have equally proved to be strong agents of disease prevention and treatment (Elekwa, 2015). These phytochemicals that have found wide therapeutic applications include: Alkaloids, flavonoids, tannins, saponins, carotenoids, etc. For instance, many alkaloids are extremely toxic and may act as neuromuscular poisons, enzyme inhibitors or membrane transport inhibitors. Alkaloids (morphine, codeine and cocaine) are used as pain killers, anaesthetics, antimalarial, stimulants, and as insecticides (Tanaka, *et al*, 2006, Elekwa, 2015). Flavonoids also play vital roles as anti-inflammatory, anti-allergic and anti-cancer roles (Formica and Regelson, 1995). Flavonoids have been shown to possess anti-hypertensive properties (Esquivel-Gutierriz, *et al*, 2013; Khanavi, *et al*, 2013). Tannins cause protein inactivation, hence used as insecticides. They also possess astringent properties. Tannins have been shown to inactivate Polio Virus, Herpes simplex and other enteric viruses (Bajaj, 1988). Saponins also serve as natural antibiotics, reducing cardiovascular diseases and reduction in cholesterol levels (Elekwa, 2005). Carotenoids are known for their antioxidant properties thereby helping the body to get rid of free radicals. They have equally been shown to possess antihypertensive properties (Eric, *et al*, 2011; James, *et al*, 2001; Magumi, *et al*, 2008).

There is no much reported works on the phytochemical components of the different parts of *Hura crepitans* (leaves, bark and roots), therefore the objective of this study was to ascertain the comparative phytochemical components of the leaves, stem bark and roots of *Hura crepitans*.

II. Materials and Methods

II.1. Plant Material Collection

Fresh leaves, stem bark and roots of *Hura crepitans* were collected from the premises of University of Nigeria, Nsukka and were identified in the Botany Unit of University of Nigeria Nsukka. The samples were air-dried under shade for one month.

II.2. Preparation of Plant Extracts:

The plant samples were pulverized using an electronic blender and 200g each of the powdered sample was dissolved using 500 ml of distilled water and allowed to stay for 72 hours. The extracts were filtered using a vacuum pump and concentrated by evaporation.

II.3. GC Conditions for Total Carotenoids:

The carotenoid extraction was carried out using the modified method Takagi, (1985).

Injection Temperature	HP 5890 Powered with Chemstation Rev. A 09.01 [1206] software
Split Ratio	20:1
Carrier Gas	Nitrogen
Inlet Temperature	250°C
Column Type	AC-5
Column Dimensions	30m X 0.25mm X 0.25um

Oven Program Initial Temperature @ 60°C first ramping @ 10°C/min for 4 minutes, maintained for 4 minutes.
Detector FID
Detector Temperature 320°C
Hydrogen Pressure 30psi
Compressed Air 40psi

II.4. GC Conditions for Total Saponins:

The extraction was carried out using the modified method of Miringquan, *et al.*, (2009).

GC Hp 6890 Powered with Chemstation Rev. A 09.01 [1206] software
Injection Temperature Split Injection
Split Ratio 20.1
Carrier Gas Nitrogen
Inlet Temperature 250°C
Column Type Capillary, DB-225ms
Column Dimensions 30m X 0.25mm X 0.25 μm.
Oven Program Initial Temperature @ 60°C for 5 minutes, First Ramping @ 12°C/min for 18 minutes, Second Ramping @ 15°C/min for 5 minutes
Detector FID
Detector Temperature 320°C
Hydrogen Pressure 30psi
Compressed Air 40psi

II.5. GC Conditions for Total Sterols:

Sterol analysis was carried out by following the modified AOAC 994.10 and AOAC 970.51 official methods.

GC Hp 6890 Powered with Chemstation Rev. A 09.01 [1206] software
Injection Temperature Split Injection
Split Ratio 20.1
Carrier Gas Nitrogen
Inlet Temperature 250°C
Column Type Hp INNO Wax
Column Dimensions 30m X 0.25mm X 0.25 μm.
Oven Program Initial Temperature @ 60°C, First Ramping @ 10°C/min for 20 minutes maintained for 4 minutes, Second Ramping @ 15°C/min for 4 minutes, maintained for 10 minutes.
Detector FID
Detector Temperature 320°C
Hydrogen Pressure 30psi
Compressed Air 40psi

II.6. GC Conditions for Phenolic Compounds:

Phenolic compounds were extracted from pulverized samples according to the method described by Ndoumou, *et al.*, (1996).

Column Rtx-5ms, 5% Diphenyl – 95% Dimethyl Polysiloxane
Column Length 30m
Column ID 0.25mm
Column Film 0.25mm
Injection Temperature 250°C
Detector Temperature 320°C
Detector FID
Injection System Splitless
Rate 1 80°C @ 6°C/min to 200°C
Rate 2 30°C/min to 280°C and maintained for 5 minutes
Mobile Phase Carrier Nitrogen
Nitrogen Pressure Column 30 psi
Hydrogen Pressure 28 psi
Compressed Air Pressure 32 psi.

II.7. GC Conditions for Flavonoids:

GC Hp 6890 Powered with Chemstation Rev. A 09.01 [1206] software
Injection Temperature Split Injection
Split Ratio 20.1
Carrier Gas Nitrogen
Inlet Temperature 250°C
Column Type Hp INNO Wax
Column Dimensions 30m X 0.25mm X 0.25 μm.
Oven Program Initial Temperature @ 50°C, First Ramping @ 8°C/min for 20 minutes, Second Ramping @ 12°C/min for 4 minutes, maintained for 4 minutes.

Detector FID
 Detector Temperature 320°C
 Hydrogen Pressure 22psi
 Compressed Air 35psi

II.8. GC Conditions For Alkaloids

Alkaloid extraction was carried out by following the modified method of Ngounou, *et al*, (2005).

GC Hp 6890 Powered with Chemstation Rev. A 09.01 [1206] software
 Injection Temperature Split Injection
 Split Ratio 20.1
 Carrier Gas Nitrogen
 Inlet Temperature 250°C
 Column Type DB – 5ms Capillary
 Column Dimensions 30m X 0.25mm X 0.25| m.
 Oven Program Initial Temperature @ 60°C for 5 minutes, First Ramping @ 10°C/min for 20 minutes, Second Ramping @ 15°C/min for 4 minutes
 Detector FID
 Detector Temperature 320°C
 Hydrogen Pressure 28psi
 Compressed Air 38psi

II.9. GC Conditions for Terpenes:

GC Hp 6890 Powered with Chemstation Rev. A 09.01 [1206] software
 Injection Temperature Split Injection
 Split Ratio 20.1
 Carrier Gas Nitrogen
 Inlet Temperature 150°C
 Column Type Hp – 5ms Capillary
 Column Dimensions 30m X 0.25mm X 0.25| m.
 Oven Program Initial Temperature @ 40°C Ramped @ 5°C/min to 200°C, Run @ 200°C for 2 minutes
 Detector FID
 Detector Temperature 300°C
 Hydrogen Pressure 22psi
 Compressed Air 28psi

III. Results and Discussion

The role of phytochemicals has been widely applied over the years especially in the treatment of diseases and as a major component of plant foods.

Gas Chromatography – Flame ionization Detector (GC – FID) is a recent technology that gives reliable evidence on the types and amounts of phytochemicals present in a given plant sample. The results of the GC – FID analysis of the leaves, stem bark, and roots of *Huracrepitans* showed that it contains varying amounts of flavonoids, carotenoids, alkaloids, terpenes, sterols, saponins and phenolic acid. The types and amounts of the different phytochemicals present are listed in the tables below:

Table 1: The comparative amounts of alkaloids present in the aqueous leaf, stem bark and roots extracts of *Huracrepitans*.

Stem Bark			Leaves			Roots		
ALKALOIDS	RT (Min)	AMOUNT [Mg/100g]	ALKALOIDS	RT (Min)	AMOUNT [Mg/100g]	ALKALOIDS	RT (Min)	AMOUNT [Mg/100g]
Choline	5.134	2.85963e-1	Choline	4.886	2.46973	Choline	5.513	3.30983e-1
Theophylline	6.012	3.3574	Theophylline	5.764	2.9675	Theophylline	6.391	3.8076
Dillapiole	6.405	4.24777e-1	Dillapiole	6.157	3.85787	Dillapiole	6.784	4.69797e-1
Angustifoline	7.001	22.7812	Angustifoline	7.153	22.3913	Angustifoline	7.38	23.2314
Sparteine	7.415	10.4103e-1	Sparteine	8.167	10.0204	Sparteine	7.794	10.8605e-1
Ellipane	8.261	8.91706e-1	Ellipane	8.913	8.52716	Ellipane	8.64	9.36726e-1
Lupanine	9.137	9.57561	Lupanine	10.189	9.18571	Lupanine	9.516	10.0258
13-Alphahydro-rhambifoline	9.432	9.10812	13-Alphahydro-rhambifoline	11.184	8.71822	13-Alphahydro-rhambifoline	9.811	9.55832
9-octadecenamide	10.902	7.46166	9-octadecenamide	12.054	7.07176	9-octadecenamide	11.281	7.91186
Dihydro-oxodemethofyhamanthamine	12.23	6.56015	Dihydro-oxodemethofyhamanthamine	13.082	6.17025	Dihydro-oxodemethofyhamanthamine	12.609	7.01035
Augustamine	12.998	5.75083	Augustamine	13.175	5.36093	Augustamine	13.377	6.20103
Oxoasoanine	13.113	7.43161	Oxoasoanine	13.365	7.04171	Oxoasoanine	13.492	7.88181
Shogal	13.312	3.22534e-1	Shogal	13.564	2.83544	Shogal	13.691	3.67554e-1
Piperline	13.475	3.02643e-1	Piperline	13.627	2.63653	Piperline	13.854	3.47663e-1
Gingerdione	13.891	3.8301e-1	Gingerdione	14.043	7.04171	Gingerdione	14.27	4.2803e-1
Capsalcine	14.114	6.71031e-1	Capsalcine	14.166	2.83544	Capsalcine	14.493	7.16051e-1
Cinchonidine	14.725	6.39698	Cinchonidine	14.077	2.63653	Cinchonidine	14.704	6.84718
Cinchonine	14.948	4.6412	Cinchonine	14.2	3.4402	Cinchonine	14.827	5.0914
Crinane-3alpha-01	15.033	8.83448	Crinane-3alpha-	14.585	8.44458	Crinane-3alpha-01	14.912	9.28468

			01					
Buphanidrine	16.048	37.2511	Buphanidrine	14.542	36.8612	Buphanidrine	14.927	37.7013
Indicine-N-oxide	17.225	9.28569	Indicine-N-oxide	15.377	8.89579	Indicine-N-oxide	15.104	9.73589
Powelline	18.067	13.4581	Powelline	16.419	13.0682	Powelline	16.146	13.9083
Undulatine	19.717	18.1745	Undulatine	16.669	17.7846	Undulatine	17.296	18.6247
Ambelline	19.864	12.6237	Ambelline	17.516	12.2338	Ambelline	17.343	13.0739
6-hydroxybuphanidrine	20.142	17.5734	6-hydroxybuphanidrine	18.294	17.1835	6-hydroxybuphanidrine	18.921	18.0236
Aeronycline	21.976	4.80104	Aeronycline	18.928	4.41114	Aeronycline	19.555	5.25124
Monocrotalline	22.803	5.82448	Monocrotalline	19.155	5.434	Monocrotalline	19.582	6.27468
6-hydroxypowelline	23.096	3.95999	6-hydroxypowelline	20.148	3.57009	6-hydroxypowelline	19.775	4.41019
Nitidine	23.236	4.33935	Nitidine	21.088	3.94945	Nitidine	20.815	4.78955
Crinamidine	24.042	20.8251	Crinamidine	22.094	20.4352	Crinamidine	20.921	21.2753
1beta,2beta-Epoxyambelline	24.832	3.06857	1beta,2beta-Epoxyambelline	22.552	2.67867	1beta,2beta-Epoxyambelline	22.139	3.51877
6-hydroxyundultine	25.066	3.32349	6-hydroxyundultine	22.618	2.93359	6-hydroxyundultine	22.165	3.77369
Epoxy-3,7-dimethoxycrinane-11-one	25.857	6.77876e-1	Epoxy-3,7-dimethoxycrinane-11-one	23.309	6.38886	Epoxy-3,7-dimethoxycrinane-11-one	23.136	7.22896e-1
Akuammidine	26.115	16.2257	Akuammidine	24.067	15.8358	Akuammidine	24.106	14.3303
Echitammidine	26.327	13.8801	Echitammidine	24.279	13.4902	Echitammidine	25.018	16.546
Voacangine	26.939	16.0958	Voacangine	24.891	15.7059	Voacangine	25.203	8.90706
Mitraphylin	27.524	8.45686	Mitraphylin	25.276	8.06696	Mitraphylin	25.847	8.90706
Camptothecin	28.068	6.40175e-1	Camptothecin	26.02	6.01185	Camptothecin	26.393	6.85495e-1
Echitamine	26.514	5.10985e-2	Echitamine	26.466	4.71995	Echitamine	27.645	5.56005e-2
Colchicines	27.066	4.76839e-2	Colchicines	27.018	4.37849	Colchicines	27.822	5.21859e-2
Emetine	27.143	3.70341e-2	Emetine	27.395	3.31351	Emetine	26.393	4.15361e-2
Tetrandrine	27.829	3.04665e-2	Tetrandrine	27.581	2.65675	Tetrandrine	28.208	3.49685e-2
Paclitaxel	29.228	5.35944e-2	Paclitaxel	28.98	4.96954	Paclitaxel	29.607	5.80964e-2
TOTAL= 379.461			Total= 362.696			Total= 398.82		

The results showed that the aqueous root extracts contain the highest amounts of alkaloids. This result conforms to the findings of Ganiyat and Mutairu, (2014), where they showed the presence of alkaloids and other phytochemicals in methanol extracts of the stem bark and leaves of *Hura crepitans*. The rich alkaloid content of the different plant extracts suggest that this plant has found a potential use as analgesic, anaesthetics, stimulants and even as an insecticide (Elekwa, 2015; Tanaka, *et al*, 2006). The alkaloid-crinamidine has been shown to be cytotoxic to various strains of Plasmodium (Likhitwitayawuid, *et al*, 1993).

Table 2: The comparative amounts of the flavonoids present in the leaves, stem bark, and roots of *Hura crepitans*.

Stem Bark			Leaves			Roots		
FLAVONOIDS	RT (Min)	AMOUNT [Mg/100g]	FLAVONOIDS	RT (Min)	AMOUNT [Mg/100g]	FLAVONOIDS	RT (Min)	AMOUNT [Mg/100g]
1 Catechi	11.251	8.40292e-2	Catechi	11.038	8.08012e-2	Catechi	11.459	8.69492e-2
1 Resveratrol	12.534	4.24841e-2	Resveratrol	12.321	3.92561e-2	Resveratrol	12.742	4.54041e-2
1 Genistein	13.009	4.11006e-2	Genistein	12.796	3.78726e-2	Genistein	13.217	4.40206e-2
1 Daidzein	13.404	4.06647e-2	Daidzein	13.191	3.74367e-2	Daidzein	13.612	4.35847e-2
1 Apigein	14.18	3.52792e-3	Apigein	13.967	3.20512e-3	Apigein	14.388	3.81992e-3
1 Butein	14.318	9.31639e-3	Butein	14.105	8.99359e-3	Butein	14.526	9.60839e-3
1 Nanasgenin	14.597	3.3007e-3	Nanasgenin	14.384	2.9779e-3	Nanasgenin	14.805	3.5927e-3
1 Biochanin	15.277	11.1826e-5	Biochanin	15.064	10.8578e-5	Biochanin	15.485	11.4746e-5
1 Luteolin	15.559	6.45571e-5	Luteolin	15.346	6.13291e-5	Luteolin	15.767	6.74771e-5
1 Kaempferol	17.025	25.1540e-5	Kaempferol	16.812	24.83125e-5	Kaempferol	17.233	25.44605e-5
1 [-]Epicatechin	17.979	3.9525e-3	[-]Epicatechin	17.766	3.6297e-3	[-]Epicatechin	18.187	4.2445e-3
1 [-] Epigallocatechin	19.328	6.23634e-3	[-] Epigallocatechin	19.115	5.91354e-3	[-] Epigallocatechin	19.536	6.52834e-3
1 Quercetin	19.868	31.3859e-5	Quercetin	19.655	31.06579e-5	Quercetin	20.076	31.68059e-5
1 Gallo catechin	20.362	7.30689e-5	Gallo catechin	20.149	6.98409e-5	Gallo catechin	20.57	7.59889e-5
1 [-] Epicatechin-3-gallate	20.739	3.32901e-3	[-] Epicatechin-3-gallate	20.526	3.00621e-3	[-] Epicatechin-3-gallate	20.947	3.62101e-3
1 [-] Epigallocatechin-3-gallate	21.473	2.65262e-2	Isorhamretin	21.26	2.32982e-2	[-] Epigallocatechin -3-gallate	21.951	2.94462e-2
1 Isorhamretin	21.743	3.86961e-3	Robinetin	21.53	3.54681e-3	Isorhamretin	22.506	6.98339e-3
1 Robinetin	22.116	6.69139e-3	Ellagid acid	21.903	6.36859e-3	Robinetin	23.196	3.19986e-3
1 Ellagid acid	22.298	2.90786e-3	Myricetin	22.085	2.58506e-3	Ellagid acid	23.409	5.76536e-3
1 Myricetin	22.988	5.47336e-3	Baicalin	22.775	5.15056e-3	Myricetin	23.581	5.96619e-3
1 Baicalein	23.201	5.67419e-3	Nobicalin	22.988	5.35139e-3	Baicalein	23.755	11.4647e-5
1 Nobicalin	23.373	11.1727e-5	Kaempferol-3,7,4-trinethyl ether	23.334	10.8499e-5	Nobicalin	24.013	4.7215e-2
1 Kaempferol-3,7,4-trinethyl ether	23.547	4.4295e-2	Quercetin-3,7,4-trinethyl ether	23.592	4.1067e-2	Kaempferol-3,7,4-trinethyl ether	23.755	3.12777e-2
Quercetin-3,7,4-trinethyl ether	23.805	2.83577e-2	Baicalin	23.334	2.51297e-2	Quercetin-3,7,4-trinethyl ether	24.031	9.14271e-2
Baicalin	23.547	8.85071e-2	Tangeretin	23.8	8.52791e-2	Baicalin	23.755	5.28077e-2
1 Tangeretin	24.013	4.98877e-2	Quercetin-	24.032	4.66597e-2	Tangeretin	24.221	4.8543e-2

Phytochemical Comparative Screening of Aqueous Extracts of the Leaves, Stem barks, and Roots

			3,7,3',4'- tetranethyl ether					
Quercetin-3,7,3',4'- tetranethyl ether	24.245	4.5623e-2	Artemetin	24.126	4.2395e-2	Quercetin- 3,7,3',4'- tetranethyl ether	24.453	4.61429e-2
1 Artemetin	24.339	4.32229e-2	Hyperoside	24.249	3.99949e-2	Artemetin	24.547	38.1882e-5
Hyperoside	24.462	37.8962e-2	Silymarin	24.359	37.5734e-5	Hyperoside	224.67	6.29388e-2
1 Silymarin	24.572	6.00188e-2	Kaempferol-3- Arabinoside	24.588	5.67908e-2	Silymarin	24.78	5.6075e-5
Kaempferol-3- Arabinoside	24.801	5.3155e-5	Quercitrin	24.939	4.9927e-5	Kaempferol-3- Arabinoside	25.009	6.2304e-5
Quercitrin	24.988	5.9384e-5	Naringin	25.104	5.6156e-5	Quercitrin	25.196	3.78316e-3
1 naringin	25.152	3.49116e-3	Isoquercetin	25.104	3.16836e-3	Naringin	25.36	3.80282e-3
Isoquercetin	25.317	3.63226e-3	Oriebtin	25.211	3.18802e-3	Isoquercetin	25.525	3.80282e-3
Oriebtin	25.424	3.63226e-3	Rutin	25.391	19.83793e-5	Oriebtin	25.632	3.92426e-3
1 Rutin	25.604	20.1607e-5	Isoorientin	25.531	2.45771e-2	Rutin	25.812	20.45273e-5
Isoorientin	25.744	2.78051e-2				Isoorientin	25.952	3.07251e-2
TOTAL= 292.2249			Total= 279.9585			Total= 303.3209		

It also shows that the root extract contains the highest amount of flavonoids. The high amounts of flavonoids present in the root suggest its use as an anti-inflammatory, anti-allergic, and anti-cancer agents (Formica and Regelson, 1995; Elekwa, 2015). They are also used as antioxidants (Donnapee, *et al*, 2014) they have equally been showed to possess antihypertensive properties owing to the Quercetin, Kameferol, Rutin and Biohannin contents (Khanavi, *et al*, 2013; Edward, *et al*, 2007, Jonathan, *et al*, 1999).

Table 3: The comparative amounts of saponins present in the aqueous extracts of the leaves, stem bark, and roots of *H. crepitans*.

Stem Bark			Leaves			Roots		
SAPONINS	RT (Min)	AMOUNT [Mg/100g]	SAPONINS	RT (Min)	AMOUNT [Mg/100g]	SAPONINS	RT (Min)	AMOUNT [Mg/100g]
Hispogenin	14.607	2.13563	Hispogenin	14.178	1.71873	Hispogenin	15.012	2.45523
Solagenin	15.835	4.93381	Solagenin	15.406	4.51691	Solagenin	16.24	5.25341
Diosgenin	16.346	0.78e-2	Diosgenin	15.917	1.1969	Diosgenin	16.751	0.4604e-2
Tigogenin	17.036	1.92382	Tigogenin	16.607	1.50692	Tigogenin	17.441	2.24342
Neochlorogenin	17.712	10.1403	Neochlorogenin	17.283	9.72339	Neochlorogenin	18.117	10.4599
Hecogenin	18.712	3.56772	Hecogenin	18.283	3.15082	Hecogenin	19.117	3.88732
Sapogenin	19.846	45.8196	Sapogenin	19.417	45.4027	Sapogenin	20.251	46.1392
Tribuloin	20.478	0.0891e-2	Tribuloin	20.049	0.506e-2	Tribuloin	20.883	0.23046e-2
Yanogenin	21.213	2.91397	Yanogenin	20.784	2.49707	Yanogenin	21.618	3.23357
Conyzorgin	22.037	1.79158	Conyzorgin	21.608	1.37468	Conyzorgin	22.442	2.11118
Saponine	23.541	54.1317	Saponine	23.112	53.7148	Saponine	23.946	54.4513
TOTAL= 126.489			Total= 121.903			Total= 130.005		

The roots also contain highest amounts of saponins. Saponins are located mainly in the cell membranes of plants. They are known for lowering blood cholesterol level, weight loss and as anti-cancer agents (Elekwa, 2015).

Table 4: The comparative amounts of phenolic acids present in the aqueous leaf, stem bark and roots of *H. crepitans*.

Stem Bark			Leaves			Roots		
PHENOLIC	RT (Min)	AMOUNT [Mg/100g]	PHENOLIC	RT (Min)	AMOUNT [Mg/100g]	PHENOLIC	RT (Min)	AMOUNT [Mg/100g]
4-hydroxy benzaldehyde	5.874	22.6718	4-hydroxy benzaldehyde	5.5	15.7723	4-hydroxy benzaldehyde	5.805	23.1396
4-hydroxy benzoic acid	9.291	19.2944	4-hydroxy benzoic acid	8.717	26.2241	4-hydroxy benzoic acid	9.022	19.7622
Methyl ester	10.382	70.5792	Methyl ester	10.208	22.8467	Methyl ester	10.513	71.047
Vanillic acid	12.819	16.7868	Vanillic acid	11.845	74.1315	Vanillic acid	12.15	17.2546
Gallic acid	13.901	88.6713	Gallic acid	12.927	20.3391	Gallic acid	13.232	89.1391
Ferullic acid	15.911	12.0994	Ferullic acid	14.737	92.2236	Ferullic acid	15.042	12.5672e-1
Capsaicin	17.937	30.6962	Capsaicin	16.363	15.6517	Capsaicin	16.668	31.164
Rosmarinic acid	18.206	123.01	Rosmarinic acid	17.286	34.2485	Rosmarinic acid	17.591	123.478
Tannic acid	21.062	22.6718	Tannic acid	19.288	15.7723	Tannic acid	19.593	23.1396
TOTAL= 383.809			Total= 301.438			Total= 387.551		

The aqueous root extracts equally showed the highest amounts of phenolic acids. Phenolic acids are important medically as antioxidants and anti-inflammatory agents (Liu, *et al*, 2013; Wang, *et al*, 2009).

Table 5: The comparative amounts of carotenoids present in the aqueous leaf, stem bark and root extracts of *H. crepitans*.

Stem Bark			Leaves			Roots		
Carotenoids	RT (Min)	Amount [Mg/100g]	Carotenoids	RT (Min)	Amount [Mg/100g]	Carotenoids	RT (Min)	Amount [Mg/100g]
Malvidin	7.992	5.008	Malvidin	7.139	3.96	Malvidin	9.858	5.421
Cavotene	9.008	46.51	Cavotene	8.155	47.558	Cavotene	10.874	46.097
Lycopene	9.911	4.9158	Lycopene	9.058	3.8678	Lycopene	11.777	5.3288
Beta-erytoxanthin	10.832	34.101	Beta-erytoxanthin	9.979	35.149	Beta-erytoxanthin	12.698	33.688
Lutein	11.073	26.602	Lutein	10.22	24.65	Lutein	12.939	23.189
Anther-xanthin	13.352	4.994	Anther-xanthin	12.499	3.946	Anther-xanthin	15.218	5.407
Asta-xanthin	14.085	6.29	Asta-xanthin	13.232	5.242	Asta-xanthin	15.951	6.703
Viola-xanthin	14.827	18.835	Viola-xanthin	13.944	19.883	Viola-xanthin	16.693	18.422
Neo-xanthin	15.475	36.01	Neo-xanthin	14.622	37.058	Neo-xanthin	17.341	35.597
Xanthophylls	12.506	33.506	Xanthophylls	11.653	34.554	Xanthophylls	6.419	33.093
TOTAL= 171.356			Total= 181.836			Total= 167.226		

The aqueous leaf extract showed the highest amount of carotenoids. Carotenoids are organic pigments found in the chloroplasts and chromoplasts of plants. They function mainly as antioxidants, anti-cancer agents and prevention of cardiovascular diseases. B-carotene- a component of carotenoid has been shown to possess strong anti-hypertensive properties (Hoon, *et al*, 2013; Eric, *et al*, 2011; Megumi, *et al*, 2008).

Table 6: The comparative amounts of sterols in the aqueous leaf, stem bark and root extracts of *H. crepitans*.

Stem Bark			Leaves			Roots		
Sterols	RT (Min)	Amount [Mg/100g]	Sterols	RT (Min)	Amount [Mg/100g]	Sterols	RT (Min)	Amount [Mg/100g]
Cholesterol	7.999	0.38132	Cholesterol	7.629	0.2177e-1	Cholesterol	8.133	0.36474
Cholestanol	16.093	1.254e - 1	Cholestanol	15.723	1.853	Cholestanol	16.227	0.5627e-2
Ergosterol	17.197	1.154e - 1	Ergosterol	16.827	1.753	Ergosterol	17.331	0.0461e-2
Campesterol	17.981	0.33445	Campesterol	17.611	0.2646e-1	Campesterol	18.115	0.0873
Stia-masterol	18.467	1.76637	Stia-masterol	18.097	1.16737	Stia-masterol	18.601	0.59029
Savenasterol	19.667	6.29703	Savenasterol	19.297	5.69803	Savenasterol	19.801	4.59061
Sitasterol	20.595	0.13872	Sitasterol	20.225	0.4603e-1	Sitasterol	20.729	0.10399e-2
TOTAL= 6.50989			Total= 2.31689			Total= 8.05969		

The aqueous root extract showed the highest amount of sterols. Sterols are important class of organic molecules. They occur naturally in plants, animals and fungi. Sterols have been shown to have high efficacy and safety in the management of blood cholesterol levels (Katan, *et al*, 2003; Harland, 2012). This shows their role in preventing cardiovascular diseases. Also, it has been shown to relieve prostate by reducing the symptoms of benign prostatic hyperplasia such as frequent urination, urgency and incomplete voiding. Plant sterols and sterols appear to be capable of selectively enhancing the activity of beneficial immune cells, while inhibiting the response of those that cause inflammation and chronic diseases (Shailendra, 2008).

Table 7: The comparative amounts of Terpenes present in the aqueous leaf, stem bark and root extracts of *H. crepitans*.

Stem Bark			Leaves			Roots		
Terpenes	RT (Min)	Norm (%)	Terpenes	RT (Min)	Norm (%)	Terpenes	RT (Min)	Norm (%)
Alpha Pinene	7.784	8.33339	Alpha Pinene	7.264	7.42319	Alpha Pinene	8.342	8.67919
Beta Pinene	9.142	30.966	Beta Pinene	8.622	30.0558	Beta Pinene	9.7	31.3118
CIS Ocimene	9.546	0.84897	CIS Ocimene	9.026	0.0612	CIS Ocimene	10.104	1.19477
Myrcene	10.355	0.1753	Myrcene	9.835	1.0855	Myrcene	10.913	0.17048
AlloOcimene	10.597	0.56338	AlloOcimene	10.077	0.3468	AlloOcimene	11.155	0.90918
Limonene	11.000	0.1356	Limonene	10.48	1.0458	Limonene	11.558	0.21021
Camphene	11.458	0.1091	Camphene	10.938	1.0193	Camphene	12.016	0.23669
Sabinene	11.834	0.3975	Sabinene	11.314	1.3077	Sabinene	12.392	0.0517
Alpha thujene	12.362	0.3257	Alpha thujene	11.842	1.2359	Alpha thujene	12.92	0.02013
Camphor	12.989	0.3965	Camphor	12.469	1.3067	Camphor	13.547	0.0507
Neral	13.252	0.4018	Neral	13.005	1.312	Neral	14.083	0.056
1, 8 - cineole	13.741	8.88795	1, 8 - cineole	13.221	7.97775	1, 8 - cineole	14.299	9.23375
Borneol	14.197	0.4084	Borneol	13.677	1.3186	Borneol	14.755	0.0626
Linalool	14.3	14.1414	Linalool	13.78	13.2312	Linalool	14.858	14.4872
Neril (geraniol)	14.651	31.2243	Nerol (geraniol)	14.131	30.3141	Nerol (geraniol)	15.209	31.5701
Alpha terpineol	14.851	0.4042	Alpaterpineol	14.331	1.3144	Alpha terpineol	15.409	0.0584
Terpinen-4-01	15.125	0.4017	Terpinen-4-01	14.605	1.3119	Terpinen-4-01	15.683	0.0559
Citronellol	15.572	0.3949	Citronellol	15.052	1.3051	Citronellol	16.13	0.0491
Ethyl Cinnamate	15.857	0.18974	Ethyl Cinnamate	15.337	0.7205	Ethyl Cinnamate	16.415	0.53554
Bomeol acetate	16.224	0.1074	Bomeol acetate	15.704	1.0176	Bomeol acetate	16.782	0.23838
Neryl acetate	16.897	0.4085	Neryl acetate	16.377	1.3187	Neryl acetate	17.455	0.0627
Geranyl acetate	17	0.4085	Geranyl acetate	16.48	1.3187	Geranyl acetate	17.558	0.0627
Tarateron	17.853	0.4069	Tarateron	17.333	1.3171	Tarateron	18.411	0.0611
Alpha amyryn	18.945	0.4094	Alpha amyryn	17.93	1.3196	Alpha amyryn	19.008	0.0636
Beta amyryn	18.945	0.4083	Beta amyryn	18.425	1.3185	Beta amyryn	19.503	0.0625
Lupeol	19.315	0.411	Lupeol	18.795	1.3212	Lupeol	19.873	0.0652

Alpha Bergamotere	19.97	0.1471	Alpha Bergamotene	19.45	1.0573	Alpha Bergamotere	20.528	0.19875
TOTAL = 88.8975			TOTAL= 64.3221			Total= 98.2341		

The aqueous root extract showed the highest amount of terpenes. Terpenes are a large and diverse class of organic compounds that are protective in function. They are major constituents of plant resins and essential oils extracted from such plants. Terpenes have found its uses both industrially and medically. For instance, they are used industrially in the manufacture of perfumes, insect repellents, cosmetics, cleaners and air fresheners. It equally has found wide application as an antiseptic because of its anti-microbial properties as well as in the treatment of fungal skin infection (Isman, 2000; Boutanaev, *et al*, 2015).

IV. Conclusion

The phytochemical screening of the aqueous leaf, stem bark and roots extracts of *Hura crepitans* have been shown that these parts of *Hura crepitans* are very rich in various phytochemicals. This plant owing to its vast phytochemicals can be evaluated further to ascertain its potential use in the treatment of various ailments as claimed by herbalists and can as well find its relevance as a potential cheap, easy accessible and available source of local raw materials.

References

- [1]. Abidi, S.L., (2001). Chromatographic analysis of plant sterols in foods and vegetable oils. *Journal of Chromatography* 935:173-201.
- [2]. Adedire, C.O. and Ajayi, O.E., (2003). Potential of Sandbax, *Hura crepitans* seed Oil for Protection of Cowpea Seeds from *CallosobruchusMaculatus*Fabricius (Coleoptera: Bruchidae) Infestation. *Journal of Plant Diseases and Protection* 110 (6):602-610.
- [3]. Bajaj, Y.P.S., (1988). Biotechnology in Agriculture and Forestry. Springer-Verlag Berlin Heidelberg New York. Pp 2-80.
- [4]. Barbieri,L; Falasca, A; Franceschi,C; Licastro,F; Rossi,F and Stripe, F., (1983). Purification and properties of two lectins from the latex of Euphorbiaceous plants *Hura crepitans* and *Euphorbia characias*. *Biochem. J.*215:433-439
- [5]. Boutanaev, A. M; Moses, T; Zi, J; Nelson, D. R; Mugford, S. T; Peters, R. J; Osbourn, A, (2015). Investigation of terpene diversification across multiple sequenced plant genomes. *Proc. Natt. Acad. Sci.* 112 (1): E81 – E88.
- [6]. Bowman,J.M.;Braxton,M.S.;Churchill,M.A.; Hellie, J.D.;Starrett, S.J.; Causby, G.Y.;Ellis, D.J.; Ensley, S.D.; Maness, S.J.; Meyers,C.D.; Sellers, J.R.; Hua, Y.;Woodsley,R.S.; and Butcher,D.J., (1997). Extraction methods for the isolation of terpenes from plant tissue and subsequent determination by gas chromatography. *Microchemical Journal* 56(1):10-18.
- [7]. David,O.M.; Ojo, O.O.; Olumekun,V.O.; and Famurewa,O.,(2014). Antimicrobial Activities of Essential Oils from *Hura crepitans*(L), *MonodoraMyristica* (GaertnDunal) and *XylopiiAethiopica*(DunalA.Rich) Seeds. *British Journal of Applied Science & Technology* 4(23):3332-3341.
- [8]. Donnapee, S; li, J; Yang, X; Ge, A. H; Donkor, P. O; and Chang, Y, (2014) CuseutaChinesis: A Systematic Review of Ethnopharmacology, Phytochemistry and Pharmacology of an important traditional herbal medicine. *J. Ethnopharmacol.* 157(c): 292 – 308.
- [9]. Duke, J.A., (1985). Handbook of medicinal herbs. CRC Press, Boca Raton, Florida. Pp 677.
- [10]. Elekwa, Iheanyichukwu, (2015). Plants: Man' Friend Indeed. 19th Inaugural Lecture, Abia State University, Uturu. Dawn Functions Nigeria Limited
- [11]. Esquivel-Gutierrez,E.R.; Noriega-Cisneros,R; Arellano-Plaza,M; Ibarra-Barajas,M; Salgado-Garciglia,R; and Saavedra-Molina,A.,(2013). Antihypertensive effect of *JusticiaSpicigera* in L-NAME induced hypertensive rats. *Pharmacologyonline Archives* 2:120-127.
- [12]. Formica, J.V. and Regelson,W,(1995). Review of the biology of quercetin and related bioflavonoids. *Food Chem. Toxicol.*33:1061-1080.
- [13]. Ganiyat, K.O. and Mutairu, B.O., (2014). Phytochemical Investigation, toxicity and antimicrobial screening of essential oil and extracts from leaves and stem bark of *Hura crepitans*. *Academia Arena* 6(5):7-15.
- [14]. Harland, J. I, (2012). Food Combination for Cholesterol Lowering. *Nutr. Res. Rev.* 25 (2): 249 – 266.
- [15]. Hoon, M.W.;Johnson, N.A.; Chapman, P.G.; and Burke, L.M., (2013). The effect of nitrate supplementation on exercise performance in healthy individuals: a systemic review and meta-analysis. *Int.J.SportExerc. Metab.* 23(5):522-532.
- [16]. Isman, M. B, (2000). Plant essential oils for pest and disease management. *Crop Protection* 19: 603 – 608.
- [17]. James.P.P.;Leach,R.;Kalamara,E.;Shayeghi,M., (2001). The World Obesity Epidemic. *Obes.Res.*4:228S-233S.
- [18]. Katan, M. B; Grundy, S. M; Jones, P; Law, M; Miettinen, T; Paoletti, R, (2003). Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. *Mayo. Clin. Proc.* 78 (8): 965 – 978.
- [19]. Keinanen, M., (1993). Comparison of methods for the extraction of flavonoids from birch leaves (*Betulapendula*Roth) carried out using high performance liquid chromatography. *J.Agric.FoodChem.*4(11)1986-1990.
- [20]. Khanavi, M.; Hajimahdipoor,H.; Emadi,F.; Khandari, N.K., (2013). Essential Oil Composition of *Thymus katschyanus*Boiss. Obtained by Hydrodistillation and Microwave Oven Distillation. *TEOP* 16(1):117-122.
- [21]. Likhitwitayawuid, K.R.;Bavovada,L.Z.; and Cordell,G.A., (1993). Revised structure of 20-hydroxytingenone and ¹³ C-NMR assignments of 22B-hydroxytingenone. *Phytochemistry* 34:759-763.
- [22]. Liu, Y; Pukala, T. L; Musgrave, I. F; Williams, D. M; Dehle, F. C; Carver, J. A, (2013). Gallic acid is the major component of grape seed extract that inhibits amyloid fibril formation. *Bio-organic and Medicinal Chemistry Letters* 23 (23): 6336 – 6340.
- [23]. Majinda, R.R., (2012). Extraction and
- [24]. Isolation of Saponins.*Methods Mol.*
- [25]. *Bio*864:415-426.
- [26]. Megumi,Y.; Masatsugu, O.; Patrick,G.H.; Sonia, S.; Lamperti, E.D.; Chung,W.; Murali, P.; Stefan, F.; and Anjana, R.,(2008). Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T-cell activation and tolerance. *Nature Immunology* 28(17):5209-5222.
- [27]. Moretti, C and Grenand, P., (1982). Les nivrees ou plantes *Ichtyotoxiques* de la Guyaner Francaise. *Journal of Ethnopharmacology* 6(2): 139-160.
- [28]. Ngounou, F.N.; Manfou,R.N.; Tapondjou,L.A.; Lontsi, D.; Kuete,V.; Penlap,V.; Etoa , F.X.;Dubois, M.A.L; and Sodengam,B.L, (2005). Antimicrobial Diterpenoid Alkaloids from *Erythrophleumsuaveolens* (Guill. And Perr.) Brenan. *Bull.Chem.Soc.Ethiop.*19(2):221-226.

- [29]. Poswal, M.A and Akpan, T.A.D., (1991). Current trends in the use of traditional and organic methods for the control of crop pests and diseases in Nigeria. *Trop. Pest Managem.* 37:329-333.
- [30]. Reiss, E.; Batista, M.T.; and Canhoto, J.M., (2008). Effect and analysis of phenolic compounds during somatic embryogenesis induction in *Feijoa sellowiana* Berg. *Protoplasma* 232:193-202.
- [31]. Shailendra, B. P., (2008). Plants Sterols and Stanols: Their role in Health and Disease. *J. Clin. Lipidol.* 2(2): 511 – 519.
- [32]. Tanaka, K.; Hara, M.; Sakamoto, T.; Higaki, Y.; Mizuta, T.; Eguchi, Y.; Yasutake, T.; Ozaki, I.; Yamamoto, K.; Onohara, S.; Kawazoe, S.; Shigematsu, H. and Koizumi, S., (2007). Inverse association between coffee drinking and the risk of hepatocellular carcinoma: a case control study in Japan. *Cancer Sci.* 98: 214-218.
- [33]. Wang, Y. J; Thomas, P; Zhong, J. H; Bi, F. F; Kosaraju, S; Pollard, A; Fenech, M; Zhou, . F, (2009). Consumption of grape seed extract prevents amyloid – beta deposition and attenuates inflammation in brain of an Alzheimer’s disease mouse: *Neurotoxicity Research.* 15 (1): 3 – 14.