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PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF ROOT, STEM BARK AND LEAF EXTRACTS OF HURA CREPITANS L. (SAND BOX TREE)

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Abstract

The aim of this study was to quantitatively determine the phytochemical constituents of root, bark and leaf extracts of Hura crepitans and antimicrobial activities of the methanol extracts on five pathogens - Escherichia coli (EC), Staphylococcus aureus (SA), Pseudomonas aeruginosa (PA), Candida albicans (CA) and Aspergillus fumigatus (AF). Results of phytochemical analysis of the methanol extracts revealed the presence of Saponins, Alkaloids, Polyphenols, Flavonoids and Phenols which were highest in leaf (11.20±1.02 mg/100g, 8.77±1.17 mg/100g, 8.21±2.13 mg/100g, 7.84±0.55 mg/100g and 7.22±2.05 mg/100g) respectively. Terpenoids were highest in bark (6.65±0.09 mg/100g) and Sesquiterpene lactones highest in root (4.92±0.14 mg/100g). Also, steroids were higher in bark (3.42±0.25 mg/100g). Antimicrobial evaluation of the extracts (leaf, bark and root) showed root extract strongly inhibited EC at (25 mg/ml concentrations, zones of inhibition (35±0.17 mm). PA showed resistance (R) to root extract. CA was inhibited at 50 mg/ml (15±0.15 mm) zones of inhibition. Bark extract strongly inhibited EC, SA, PA and CA but not AF. Zones of inhibition at 25mg/ml concentration of Bark extract was 35±0.01 mm (EC), 18±0.12 mm (SA), 38±0.12 mm (PA), 39±0.11 mm (CA) and 13±0.19 mm (AF). Leaf extract strongly inhibited EC, SA, PA, CA and AF at 100 mg/ml. Leaf extract concentration zones of inhibition were, 21±0.19 mm (EC), 21±0.25 mm (SA), 25±0.23 mm (PA), 20±0.12 mm (CA) and 14±0.14 mm (AF). 50 mg/ml concentration of leaf inhibited SA (zone of inhibition 27±0.20 mm), PA (zone of inhibition 22±0.19 mm) and 12±0.10 mm for CA and AF respectively. These results show that the extracts of Hura crepitans possess appreciable antimicrobial and possible pesticide potentials which are attributable to their phytochemical constituents.

Keywords: Hura crepitans, phytochemical, Escherichia coli (EC), Staphylococcus aureus (SA), Pseudomonas aeruginosa (PA), Candida albicans (CA) and Aspergillus fumigatus (AF)

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Introduction

Hura crepitans L. (sandbox tree) also known as passum wood, monkey's dinner bell, monkey's pistol, monkey-no-climb, ochoo, arboldel Diablo, acacu, habillo, ceiba de' leche, dynamite tree, ceibablanca, assacu, pasentri and Jabillo (Taylor, 1998; Clarke, 2000; PIER, 2005; TBA, 2010; Lusweti et al., 2011) belongs to the family of Euphorbiaceae. Hura crepitans Linn and is synonymous with Hura brasillensis Linn (Swaine and Beer, 1977; Morley, 2000). Hura crepitans is native to the tropical regions of North and South America in the Amazon Rain forest. Places of its naturalization include North Australia and East Africa from there it invaded parts of Tanzania. It was introduced to West Africa, and planted as an exotic ornamental plant for shade.

In Calabar - Nigeria, the tree is used as an aesthetic plant to beautify the city and provide shade. It has spread to nearby Local Government areas and villages. It grows best on sandy or clay moist soils (pH 5-8), mostly in the forested shades (PIER, 2005). The yellowish milky juice from the bark, leaf and root can be used to poison fishing darts (Jones, 2007). The juice contains two lectins which have haemagglutinating activity and inhibits protein synthesis (Moris et al., 2004). The seeds are emetic and when green and fresh are highly very purgative. Oil extracted from dried seed is also used as a purgative (Fowomola et al., 2006). Its pale yellow or brown soft wood is used for furniture under the name Hura. In Suriname, the sandbox tree often can be found in nearly pure stands on moist sandy loam soil in the flat coastal region. The leaves are used against eczema, and other skin diseases (Moris et al., 2004; Mwine and Van Damme, 2011).

Tropical climate is favorable to all kinds of pests including insects, microorganisms and mycotoxins, reptiles and predatory birds. Burning of the wood has been reported to repel insects (Liach, 1971; Chudnoff, 1984; Wangaard and Mushler, 1992; Clarke, 2000). The use of synthetic pesticides for the control of these pests is unsafe and has deleterious effect on humans and food crops. Natural propensities for pesticidal activity are rare. The search for such plants as major and new candidates is ongoing as their uses are safer. *Hura crepitans* is a plant that may possess these properties (Fagbemi and Adebowale, 2000; Raton, 2003; Alves *et al.*, 2012). Therefore, the discovery of natural sources

with the potential to control pests in the environment is worth being studied and investigated and is the basis of these experiments.

Methods

Collection and Extraction of *H. crepitans* (root, bark and leaf)

Fresh and mature leaves of Hura crepitans were harvested from the open pavilion of the University of Calabar, Nigeria. The leaves were authenticated by a taxonomist in the Department of Botany, University of Calabar, Nigeria and voucher specimen (UNICAL/BCM/2017/HC-11/CNL). was deposited in the herbarium of the Department. The stem bark was cut off using chisel and knife and root dogged and cut out with cutlass. The samples were air-dried at ambient temperature for two weeks. The dried plant parts were pulverized using a laboratory mechanical grinder. Twenty grams of the dried leaf, bark and root powder were macerated in 100 ml of methanol and dichloromethane (1:1). The extracts were filtered out with chess cloth and later, filter paper. The filtered extracts were then evaporated in vacuo using a rotary evaporator (Büchi, Switzerland) to obtain an oily brown, green and dark extracts. The dry extracts were placed in amber-coloured glass bottles and stored in a refrigerator (4 °C) until use. The extracts were used for phytochemical analysis and to measure antimicrobial activities (Minimum Inhibitory Concentration - MIC and sensitivity tests).

Quantitative Phytochemical Screening

Phytochemical analysis of the methanol extracts was carried out using standard procedures as described by Harbone (1973) and Trease and Evans (1989).

Antimicrobial analysis

Three human pathogens: one Gram-positive Staphylococcus aureus (SA): (ATCC: 27856) (Wilson and Stuart, 1965; Stich, 1932), and two Gramnegative Escherichia coli (EC), Pseudomonas aeruginosa (PA): (ATCC: 27856), were used for the antibacterial assay. One yeast, Candida albicans (CA): (MTCC: 227) and one mold, Aspergillus fumigatus (AF): (MTCC: 227) were used for the antifungal assay. All the organisms were local isolates from the Laboratory bacterial stock of the Department of Microbiology, University of Calabar, Nigeria. Three to five identical colonies from stored slopes of microorganisms (bacteria and fungi) were lifted

with a sterile wire loop and transferred into a 5ml single strength nutrient broth (Biochemica, Spain) contained in well labeled screw cap bottles for each bacterium and fungus respectively. The bottles were well shaken and incubated at room temperature for 24-48 h for bacteria and 72 h for fungi. 15ml of melted and cooled nutrient agar (Himedia Laboratories, India) and potato dextrose agar (Himedia Laboratories, India) were added to 0.2 ml of 1 in 100 dilutions of bacterial and fungal cultures respectively in sterile Petri dishes. The contents were mixed.

After the agar in each plate had solidified, six wells of 5 mm each were bored in each plate using an aseptic cork borer. 0.1 ml of plant extracts at varying concentrations (3.18 mgml⁻¹, 6.25 mgml⁻¹, 12.5 mgml⁻¹, 25 mgml⁻¹, 50 mgml⁻¹, 100 mgml⁻¹) as well as the standard antibiotic (Ampicillin) solution was loaded into the wells. Control experiments were set up with confirmed concentrations as confirmatory test for bacterial and fungal assays respectively. The plates were incubated at 37 °C for 24 h for bacteria and 72 h for fungi. All inoculation undertaken under procedures were aseptic conditions. According to pharmacological and biometric specifications, the antimicrobial studies were done in triplicates. With the aid of a transparent ruler the diameters of zones of inhibition around the wells were measured in mm for all the three replicates and the average of the three measurements was calculated as an indication of activity. The minimum inhibitory concentration (MIC) of the plant extracts was determined using the agar well diffusion method as described by (Sahm and Washington, 1990). The test tube with the concentration of plant extract at which no detectable growth was observed was considered as the MIC (LD_{50}).

Results

Table 1 shows the results of the quantitative phytochemical contents of *Hura crepitans*. The table also shows the variation in phytochemical concentration between different parts of the plant (leaf, bark and root). Table 2 shows the anti-nutrient composition of the plant which also varied between the different parts of the plant.

Table 3 shows the antimicrobial effects of different concentrations of different parts of the plant. The table also shows the zones of inhibition

of each concentration and each plant part. Table 4 shows the minimum inhibitory concentration (MIC) and abbreviations for microbes used for the study.

Discussion

Quantitative phytochemical analysis revealed significant (p<0.05) concentration of prominent secondary metabolites. Variation of secondary metabolites was observed in the leaf, bark and root. The concentration of phenolics, saponins and flavonoids were observed to be higher in leaf than in bark and root, while the concentration of sesquiterpene lactones, steroid and glycosides were higher in bark and root of the plant. This trend had been reported by Fenwick and Oakenfall (1982). Saponins in leaf, bark and root gave 11.20±1.02 mg/100g, 7.41±1.30 mg/100g and 5.27±0.50 mg/100g respectively, supporting the report on saponins from food plants earlier reported in the survey by Fenwick and Oakenfall (1982). The concentration of saponins in Hura crepitans, may contribute to the antimicrobial inhibition by extracts of the plant. Alkaloid concentration in the plant was also high, as 8.77±1.17 mg/100g in leaf, 7.25±0.45 mg/100g in bark, and 7.08±0.62 mg/100g in root. The total polyphenols concentration in leaf, bark and root were at 8.21±2.13 mg/100g, 5.96±0.91 mg/100g and 5.55±0.72 mg/100g respectively. Polyphenols are generally strong antioxidants and antimicrobial (Appel, 1992; Fowomola and Akindahunsi, 2007). Their high concentration in Hura crepitans may have contributed to the significant inhibition of the human pathogens tested in vitro (Boberg, 1990; Appel, 1992; Ahmed et al; 2006 and Okigbo et al., 2009). The flavonoids concentrations estimated to be 7.84±0.55 mg/100g for leaf, 6.33±0.47 mg/100g for bark, and 5.89±0.77 mg/100g for root extract. Phenolic compounds in leaf, bark and root were estimated to be 7.22±2.05 mg/100g, 6.43±1.19 mg/100g and 5.05±0.55 mg/100g. The combined antioxidative and antimicrobial potency of phenolics, polyphenolics and flavonoids in plant extracts have been variously reported (Dix, 1979; Elliger et al., 1981; Blakeman and Atkinson, 1981). Terpenoids revealed 6.29±1.31 mg/100g in leaf, 6.65±0.09 mg/100g in bark, and 5.21±0.02 mg/100g in root. They may have also contributed to the antimicrobial potency of the plant. Fowomola et al., (2006) had earlier reported the variation of antimicrobial activities of *H. crepitans* seed oil. Sesquiterpene lactones in leaf, bark and root showed significant levels (4.71±0.27 mg/100g, 4.85±0.64 mg/100g and 4.92±0.14 mg/100g respectively). Sesquiterpene lactones are known to be highly effective insecticides and antimicrobial agents, and may also contribute to the antimicrobial potency of *Hura crepitans*. Steroid content estimated revealed 3.42±0.25 mg/100g in leaf, 3.29±0.20 mg/100g in bark and 3.14±0.07 mg/100g in root.

In vitro antimicrobial evaluation of the extract of the leaf, bark and root was carried out using five human pathogens including, Escherichia coli (EC), Staphylococcus aureus (SA), Pseudomonas aeruginosa (PA), Candida albicans (CA) and Aspergillus fumigatus (AF) by the agar well diffusion method. Root extract strongly inhibited EC at concentrations of 25 mg/ml and 50 mg/ml, giving zones of inhibition as 35±0.17 mm and 21±0.16 mm respectively. Zone of inhibition for SA at 100 mg/ml was 39±0.1 mm, and at 50 mg/ml (29±0.13 mm), and 25 mg/ml was 35±0.12 mm. PA was resistant (R) to root extract. CA was inhibited at 50 mg/ml (zone of inhibition 15±0.15 mm) and 100 mg/ml (zone of inhibition, 21±0.14 mm). AF was strongly inhibited at 100 mg/ml (zone of inhibition, 36±0.17 mm) and 25 mg/ml (zone of inhibition, 27±0.19 mm). These profiles of zone of inhibition by root extract at the three concentrations confer results determined for standard (Broad spectrum) drug (Ampicillin). Bark extract strongly inhibited EC, SA, PA and CA but less so for AF. Zones of inhibition at 25 mg/ml concentration of Bark extract was 35±0.01 mm (EC) 18±0.12 mm (SA), 38±0.12 mm (PA), 39±0.11 mm (CA) and 13±0.19 mm (AF), which compared favorably with Ampicillin (zone of inhibition, 44±0.22 mm at 25 mg/ml). Leaf extract strongly inhibited EC, SA, PA, CA and AF at 100 mg/ml. Leaf extract concentration gave zones of inhibition as, 21±0.19 mm (EC), 21±0.25 mm (SA), 25±0.23 mm (PA), 20±0.12 mm (CA) and 14±0.14 mm (AF) compared to Ampicillin which inhibited all test organisms very significantly (P <0.05) at all concentrations. 50 mg/ml concentration of leaf inhibited SA (zone of inhibition 27±0.20 mm), PA (zone of inhibition 22±0.19 mm) and 12±0.10 mm for CA and AF. Results of phytochemical analysis showed increase active metabolites in

concentration from leaf to root, the reverse for lectins and antimicrobial activity.

The MIC showed no significant difference between the extracts when compared with Ampicillin although the leaf extract had better activities against EC (3.18) and SA (3.18) than Ampicillin (6.26).

Hura crepitans plant was found to contain toxicants, processing through fermentation may enhance its utilization as an alternative source of food. In addition, it could also be used as a natural source of antimicrobial agents. The overall performance of the extracts showed significant inhibition of both bacteria and fungi strains by Hura crepitans, confirming its probable potentials as an anti-microbial agent and may be, as a pesticide and an allelopath against weeds.

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Table 1: Phytochemical Constituents of Leaf, Bark and Root of Hura crepitans (mg/100 g)

Name of Compound	Leaf	Bark	Root
Lectins	1.82±0.24	1.91±0.05 ^a	1.98±0.11 ^{ab}
Saponins	11.20±1.02	7.44±1.30 * ª	5.27±0.50* ^{ab}
Alkaloids	8.77±1.17 *	7 . 25±0.45* ^a	7.08±0.62* ^b
Flavonoids	7.84±0.55	6.33±0.47* ^a	5.89±0.77* ^{ab}
Carotenoids	2.49±0.22	1.91±0.05* ^a	1.55±0.02* ^{ab}
Limonoids	1.25±0.07	1.11±0.12	1.05±0.06
Volatile Organic	2.46±0.45	2.62±0.15 ^a	2.59±0.17
Compds	6.29±1.31	5.56±0.09* ^a	5.21±1.02* ^b
Terpenoids	3.42±0.44	3.17±0.18 ^a	2.72±0.15* ^{ab}
Sterols	3.42±0.25	3.29±0.20	3.14±0.07
Steroids	4.71±0.27	4.85±0.64	4.92±0.41
Sesquiterpene	2.64±0.42	2.59±0.27	2.69±1.13 *
lactones	2.29±0.08	1.82±0.01* ^a	1 . 77±0.22* ^b
Cardiac glycosides	2.18±0.18	1.69±0.07ª	1.71±0.12
Anthraquinones	7.22±2.05	6.43±1.19* ^a	5.05±0.55* ^b
Anthocyanins	8.21±2.13*	5.96±0.91* ^a	5.55±0.72 * ^b
Phenolic Compounds			
Total Polyphenols			

Values are presented as Mean±SEM, n=3.

b=p<0.05 vs Leaf

Table 2: Anti-nutrient composition of Leaf, Bark and Roots of Hura crepitans (mg/100g)

Name of Compound	Leaf	Bark	Root
Tannins	2.21±0.09*	1.91±0.04ª	1.02±0.02* ^{ab}
Oxalates	5.61±0.65*	4.92±0.42 ^a	4.70±0.12 ^b
Phytates	2.27±0.55*	1.14±0.03 ^a	1.01±0.04 ^b
Cyanates (as HCN)	3.77±0.44*	3.82±0.61ª	5.12±0.42 ^b

Values are expressed Mean±SEM, *significantly different from leaf at p<0.05; a=p<0.05 vs Leaf b=p<0.05 vs Leaf

^{*}significantly different from leaf at p<0.05; a=p<0.05 vs Leaf

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Table 3: Zone of Inhibition of test organisms (mm) of Methanol leaf, stem bark and root Extracts of Hura crepitans

Plant Extract	Organisms														
conc. mg/ml	FC FC		SA		PA		CA		AF						
	100	50	25	100	50	25	100	50	25	100	50	25	100	50	25
Root	14±0	21±0	35±	39±	29±	35±	13±0	10±	R	21±0	15±0	R	36±	11±0	27±
	.10	.16	0.17	0.10	0.13	0.12	.18	0.12		.14	.15		0.17	.11	0.19
Bark	36±	24±	35±	30±	21±0	18±	14±0	31±0	38±	26±	31±0	39±	19±	16±	13±0
	0.19	0.11	0.10	0.23	.25	0.12	.24	.21	0.12	0.13	.11	0.10	0.19	0.16	.19
Leaf	21±0	R	R	21±0	27±	13±0	25±	22±	14±0	20±	12±0	10±	14±0	12±0	R
	.19			.25	0.25	.19	0.23	0.19	.23	0.12	.10	0.12	.14	.19	
Ampicillin	28±	27±	25±	25±	25±	20±	32±	30±	30±	30±	35±	30±	35±	30±	30±
	0.05	0.11	0.15	0.14	0.11	0.13	0.14	0.09	0.08	0.12	0.10	0.08	0.04	0.05	0.02

Mean±SEM, n=3, R= Resistant

Table 4: Minimum Inhibitory Concentration (MIC) of Methanol leaf, stem bark and root Extract of Hura crepitans

ORGANISMS/MIC (mg/ml)								
Extract	EC	SA	PA	CA	AF			
Stem Bark	6.26	12.5	3.18	6.26	12.5			
Leaf	3.18	3.18	6.26	12.5	3.18			
Root	6.26	-ve	3.18	3.18	3.18			
Ampicillin	6.26	6.26	3.18	3.18	3.18			

EC = Escherichia coli, SA = Staphylococcus aureus, PA = Pseudomonas aeruginosa, CA = Candida albicans, AF = Aspergillus fumigatus.

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