African Scientist Vol. 25, No. 4 December 31, 2025
Printed in Nigeria
1595-6881/2023 $80.00 + 0.00

https://africansciientistjournal.org

afs2025045/26402

Preliminary Phytochemical Screening of *Hura crepitans* Leaf, Stem Bark, and Root Aqueous Extract

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(Received November 9, 2025; Accepted in revised form November 19, 2025)

ABSTRACT: This study investigate preliminary phytochemical analysis of aqueous *Hura crepitans* leaf, stem bark, and root. The result the phytochemical screening reveal that flavonoids, polysteroids, terpenoids, tannins, glycoside, alkaloids, phelonics, and saponins are present on leaves, stem bark, and root of *Hura crepitans* plant. These secondary metabolites are more concentrated at the root, followed by the stem bark; while the leaves has the least concentration and they exhibit a strong physiochemical and pharmacological effects as they traditionally employed in herbal medicines, they also pose reductive and stabilizing effect in nanoparticle synthesis where they act as natural reducing and capping/stabilization agents by donating electron to metal ions.

Keywords: *Hura crepitan*, Flavonoids, Secondary metabolites, Herbal medicines

Introduction

Sandbox tree is a tropical American angiosperm plant with the scientific name *Hura crepitans*. It belongs to a spurge family known as *Euphorbiaceae.* in the rainforest tropical regions, these plants are ornamentally planted to provide shade for recreational purposes, both in cities and villages. (Owojuyigbe, *et al*., 2020). Traditionally, its use has been found as a medicinal plant in several areas as anti-inflammatory, antimicrobial, hepatoprotective agents, emetic, purgative, and leprosy treatment (2). These phytochemicals, also known as secondary metabolites, are used in many areas. Hurina and crepitina, also kown as toxalbumins has been reported to make this plant toxic (Falasca, *et al* 1980; Jaffé, *et al* 1943). They were also found to contain diterpene hura-toxin, which is toxic in their sap. They contain glucosamine and lectin in their seed, which possess mitogenic and Haemagglutinating characteristics (Kawazu, 1972; Falasca, *et al* 1980). The stem-back hexane extract of this tree is most toxic, the ethyl acetate extract of its' leaves was least toxic (Jernigan, *et al* 2009). This plant embeds many phytochemicals such as alkaloids, phenolics, and steroids in its leaves and stems. In Greek, the word phyto, means plant, thence. phytochemical means plant chemical. They are natural chemicals or bioactive compounds produced by plants that are health beneficial to man and help plants in their defense mechanism against exposure to UV, stress, environmental hazards, drought, pollution, and an attack of pathogens. (Saxena, *et al* 2013,Linus, 2025). Plants also uses phytochemicals as nutrients, as well as reproduction and survival-enhanced metabolites. (Frank, *et al* 2020; Hassan, *et al* 2020) Their significant dietary intake is helpful in protecting human life, and over 4,000 phytochemicals have been classified in functions, but about 150 of them have been studied fully. These bioactive molecules posses several functions as those known to be essential nutrients from the human diet, which has physiological role. (Frank, *et al* 2020). Some phytochemicals are traditionally used as medicines, salicin from the white willow tree has pain-relieving and anti-inflammatory roles, and is used in the aspirirn synthesis. (Sneader, 2000; Landau, 2010). Paclitaxel, extracted from toxic English yew, has been used as an anti-cancer, and some dietary fibers that are non-digestible, which are considered to be phytochemicals reduces risk of some cancer types and coronary heart disease. (Linus, 2025). Phytochemicals from sandalwood, like sesquiterpene santolos, also find their use in the fragrance industry (Ellena, 2022). Some are natural poisons known as phytoxins (Iwasaki, 1998; Bjeldanes & Shibamoto 2009), even at small concentrations, aristolochic acid causes cancer(Shaw, 2010). Tropane alkaloids from *Atropa belladonna* are poisonous (Wink, 1998; Timbrell, 2005), Whereas some are known anti-nutrients, they prevent nutrient absorption and assimilation (Popova & Mihaylova 2019).In general, phytochemicals like phenolic compounds, flavonoids, alkaloids, tannins, saponins, terpenoids etc has been reported to act as both reducting and stabilizing agents in nanoparticles synthesis (Shahzadi, *et al*., 2025; Elmehalawy, *et al*., 2025; Thakur, S., et al., 2025; Oladipupo, *et al*., 2025; Selvam, *et al* 2026).

Materials and methods

Material: Hura crepitans leaf, electric blender, water bath, beakers, volumetric flask.
Reagent/chemicals: Sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), acetic anhydride ($(CH_3CO)_2O$), iodine crystal (I), potassium iodide (KI), mercuric chloride ($HgCl_2$), hydrochloric acid (HCl), glacial acetic acic (CH_3COOH), chloroform ($CHCl_3$), distilled water. All chemicals used are of analytical grade and they were used without purification.
Methods
Collection and identification of plant materials: Fresh mature leaves, stem bark, and root of the *Hura crepitans* plant were collected from Fatilami Park, Abakaliki, Ebonyi State Nigeria. They were identified, confirmed and authenticated in the Department of Biology, Alex Ekwueme Federal University Ndufu-Alike by taxonomist and it was given accession number AE-FUNAI 0031.
Sample preparation: The samples were washed with tap water and then rinsed in distilled water to get dirt off, and then dried in the air in a dust-free room at room temperature. A laboratory electric blender was used to pulverize the dried samples into a fine powder to have a uniform size of particles and was kept in different air-tight container for future use.
Aqueous extraction: The phytochemicals were extracted by adding 200 g each of leaf, stem bark, and root powder to 500 cm^3 of distilled water in different beakers and allowed to stay for 72 hours. The solution obtained were filtered with a Whatman filter to get a clear aqueous extract and were concentrated to 50 cm^3 each by evaporation, 10 cm^3 each of it were kept in freezer for use; the other 40 cm^3 each were dried by evaporation to dryness and kept in air-tight container for analysis (Adindu, *et al*., 2016).
Preparation of reagent:

- NaOH, 2 $moldm^{-3}$ of NaOH was prepared by dissolving 8 g of NaOH in distilled water and make up to mark in 100 cm^3 of distilled water
- $FeCl_3$, 0.1 g of $FeCl_3$ (0.1%) was dissolved in 100 cm^3 of water.
- Mayer's reagent, is prepared by dissolving 5 g of potassium iodide (KI) and 1.36 g of mercuric chloride ($HgCl_2$) in distilled water and transfered to 100 cm^3 volumetric and make up to the mark with distilled water
- Wagner's reagent, is prepared by dissolving 2 g of potassium iodide and 1.27 g of iodine, transferred them to 100 cm^3 volumetric and make up to the mark with distilled water
- 2 $moldm^{-3}$ of HCl by diluting with $C_1V_1=C_2V_2$ after calculating C_1 with $M = SGx\% \frac{purityx10}{MM}$
- 2 $moldm^{-3}$ of H_2SO_4 by diluting with $C_1V_1=C_2V_2$ after calculating C_1 with $M = SGx\% \frac{purityx10}{MM}$

Phytochemical screening: The phytochemical screening of the aqueous extract of *Hura crepitans* leaves, stem bark, and root followed the testing standard of (Harborne, 1998; Peiris, *et al*., 2023; Maheshwaran, *et al*., 2024), showing the presence or absence of phytochemicals.
Test for flavonoids: (Alkaline Reagent Test) Extract + 3 drops of 2 mol/dm^3 NaOH ,Intense yellow colour, + 2 mol/dm^3 (dil) HCl turn Colourless indicates the presence of flavonoids.
Test for steroids: (Acetic Anhydride Test) 0.5 g of extract + 2 cm^3 acetic anhydride shows Violet + 2 cm^3 H_2SO_4 shows violet blue clouration indicates the presence of polysteroids.
Test for terpenoids: (Salkowski Test) 0.2 g of extract + 2 cm^3 $CHCl_3$ + 3 cm^3 conc. H_2SO_4 was carefully added to form a layer. Redish-brown colouration at the interface shows the presence of terpenoids.
Test for tannins: (Ferric Chloride Test) 5 g sample + ethanol (abs) filter gives green colour, (b)1 cm^3 of extract + 2 cm^3 distilled H_2O gives blue-green, (c) + 2 drops 0.1% $FeCl_3$ gives Blue-black precipitate indicates the presence of tannins
Test for glycosides: (Keller-Killiani Test) 0.5 g of extract + CH_3COOH + $FeCl_3$ + H_2SO_4 gives a brown ring at the interface precipitate indicates the presence of glycosides.

Test for alkaloids: Evaporated 2 cm^3 of extract to dryingness (b) Residue + 5 cm^3 2 mol/dm^3 HCl + filter gives Brownish. (i) filtrate from (b) 2 drops mayer's reagent gives cream precipitate, (ii) 2 cm^3 filtrate from (b) + 2 drops of Wagner's reagent gives brown-coloured precipitate shows the presence of alkaloids.
Test for phenolics: (Ferric Chloride Test) 1 cm^3 of extract + 4 drops of $FeCl_3$ gives a bluish-black colouration shows the presence of phenolics.
Test for saponins: (Frothing Test) 1 g of sample + 5 cm^3 of water, foaming (frost) appears indicates the presence of saponins.

Result and Discussion

The result of the phytochemical screening of Hura crepitans leaves, stem bark, and root was as presented in Table 1

Table 1: Qualitative phytochemical analysis of Hura crepitans leaf, stem bark, and root.

S/N	Metabolite	Leaves	Stem bark	Root	Observation/Test result
1	Flavonoid	+	++	+++	Intense yellow colour, turn Colourless
2	Polysteroids	+	++	+++	Violet, turn Violet blue
3	Terpenoids	+	++	+++	Redish-brown colouration at Interface
4	Tannins	++	++	+++	Green, turn Blue-green, turn Blue-black ppt
5	Glycosides	+	+	++	Bluish-green, turn to brown ring at interface
6	Alkaloids	++	++	+++	Brownish, turn Creamy ppt, turn Brown-coloured ppt
7	Phenolics	+	++	+++	Bluish-black colouration
8	Saponins	+	++	++ +	Foaming (frost) appears

Key +: present, ++: present in big quantities, +++: present in bigger quantities.

Reagents and color/precipitate observations were used to indicate the presence of the phytochemicals (Nortjie, *et al*., 2022; Singh, *et al*., 2022). It shows that the leaves, stem bark, and root extract of *Hura crepitans* contain flavonoids, steroids, terpenoids, tannins, glycosides, alkaloids, phenolics, and saponins. Their amounts varies depending on plant parts, roots in most cases has the highest concentrations of the phytochemicals, followed by the stem bark; while the leaves has the least amount of all of them as was observed by their test affinity (Adindu *et al.,* 2016). Flavonoids, tannins, alkaloids, and saponins has been reported to possess physiological action and exhibit medicinal properties (Ibrahim *et al.,* 2017). He also reported that terpenoids exhibit good antimicrobial activity and is important in herbal therapeutic medicines. Tannins in the other hands was reported to exhibit anticancinogenic property because it prevents the growth of tumors. They show properties as being anti-neoplastic and cytotoxic (Moses *et al*., 2019), He as well reported that saponons has cleansing effect of detergents, and have been effectively used in histochemistry staining, wight-loss, antioxidant, anti-inflammatory, hypercholesterolaemia, anticancer, anti-fungal, contraceptive, cardiotonics, and hyperglycaemia. Phenolics, flavonoids, alkaloids, terpenoids found their use in nanoparticle synthesis owing to their reducing and capping effect of them. Flavonoids and polyphenols are the primary reducing agents, terpenoids is the auxilarry reducing agent, they act as natural reducing agents by donating electrons to metal ions, leading to nanoparticle formation, whereas alkaloids and proteins caps/stabilize them (Selvam *et al*., 2016; Shahzadi *et al*., 2025). Phytosterols are secondary metabolites that helps in membrane, stabilization, antimicrobial, cholesterol reduction, anticancer, antioxidant, cardioprotective effects, and anti-inflammatory activity (Shen *et al*., 2024; Khallouki, *et al*., 2024).

Conclusion

The result of the preliminary phytochemical screening of aqueous leaf, stem, and root extract of *Hura crepitans* shows that Flavonoids, polysteroids, terpenoids, tannins, glycosides, alkaloids, phenolics, and saponins are present in these parts of the plant. This plant, due to its immense phytochemicals exhibit pharmacological and physiochemical actions. isolation, purification, and incorporation of the right component in herbal medicine will impact good health in humans. The extract has shown its reductive and stabilization effects in nanoparticle synthesis.

Acknowledgement

The authors acknowledge the staff of Alex Ekwueme Federal University, Ndufu Alike, for their assistance with their school laboratory work to achieve this success.

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