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Preliminary evaluation of the Phyto-physicochemical Properties of Leaves of *Rauwolfia vomitoria* Afzel

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Abstract

Objectives: In Nigeria, *Rauwolfia vomitoria* (RV) has been used traditionally for the management of various health malaises including cancer. Some studies has also reported its medicinal functions including being anti-inflammatory, analgesic, and be useful for cardiovascular disorders. This study, therefore aimed at investigating the active agents implicated in cancer management through evaluating its phyto-physicochemical properties.

Material and Methods: Fresh leaves of RV were collected, air-dried and milled to powder form. This powdered form was implored in proximate analisys and elemental screening while the remaining was subjected to crude ethanolic extraction for pharmacognostic studies. Proximate investigated were moisture, ash, fat, and crude fibre. The phytochemicals screened included alkaloids, tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids, and cardiac glycosides.

Results: Proximate analysis revealed that moisture content had the highest concentration (6.40%), followed by crude fiber (4.25%). Ash content was extremely insignificant (<0.001%), while fat was not detected (0.00%). Elemental quantification revealed that iron was more prevalent in the plant (540.81mg/kg), followed by manganese (31.04mg/kg), zinc was observed to be 29.69mg/kg, chromium; 24.22mg/kg, copper; 8.16mg/kg, nitrogen; 0.35mg/kg. Lead, nickel, and cadmium had the same concentration of <0.001mg/kg ranking the lowest among the heavy metals.

Conclusion: Pharmacognostic screening confirmed the absence of tannin and anthraquinone but presence of cardiac glycosides, steroids flavonoids alkaloids, steroids, and terpenoids. The presence of terpenoids and flavonoids may serve as a strong indication of RV being an anti-cancer plant. However, the synergistic effect of these phytochemicals with manganese, zinc, and copper may be responsible for the medicinal potential of RV. This investigation, therefore, validates the folkloric claims attributed to RV as possessing vital medicinal values.

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Introduction

Herbs and medicinal plants are vital substances that have been used for several years in curing and managing several health malaise, disease prevention, flavouring and food preservation [1]. Active agents engendered during secondary metabolism have been reported to be responsible for the pharmacological properties of these medicinal plants [2]. Sivanandham [3] reported that approximately 50,000

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metabolites have been found in plants with a projected expectation to attain 200,000.

Modern research has grasped its success and has employed numerous natural remedies known to ancient civilizations and utilized in modern pharmacotherapy for millennia [4]. Medication derived primarily from medicinal plants traditionally has been of immense importance in protecting humans from diseases. These traditional pharmaceuticals are gaining accelerated popularity due to their affordability, availability, effectivity and lack of substantial adverse effects [5, 6].

Rauwolfia vomitoria Afzel is widely distributed in most tropical forest of Africa, Asia, the pacific and South America. In Nigeria it is locally referred to as 'Asofeyeje' in Yoruba, 'akanta' in Igbo and 'wada' in Hausa [7]. It belongs to the family Apocynaceae, a perennial plant that can attain a height of 15m high [8]. It possess oval leaves and clusters of tiny flowers with red berries (Asoro et al 2018). RV has been used in folklore as antimalarial, antipyretic, purgative, emetic, against snake venom and nervous disorders [8]. This study, therefore aimed at investigating the active medicinal agents inherent in this plant with a view to determining the ingredients implicated in cancer management through evaluating its phyto-physicochemical properties.

Materials and Methods

Sample Collection

Fresh leaves of the plant *Rauwolfia vomitoria* were procured from Mushin area of Lagos State in the month of June, 2022. The leaves were air-dried and milled into powder form, then kept in an air-tight container until required for analysis.

Plant identification and authentication

The plant leaves were identified in the Herbarium Unit of Department of Botany, University of Lagos with authentication voucher N0. 0164.

Preparation of Plant Extract

The powder leaves of *Rauwolfia vomitoria* weighing 391.8grams was soaked in 1.47 litres of ethanol for 4 days (96 hours) to get the extract. The extract was filtered and filtrate placed in regulated oven at 40 ± 1^0C. After evaporation, 24.94grams of crude extract of RV was derived and stored in refrigerator.

Phytochemical Analysis

Preliminary phytochemical screening was carried out to detect the following parameters, alkaloids, tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides.

Tannins. This was evaluated according to Parekh and Chanda [9] briefly, 2ml of extract was added to 2ml of 5% $FeCl_3$ and observed for formation of yellow brown precipitate.

Alkaloids. This was screened according to Ogunyemi [10] 1.5ml of 1%HCl was added to 2 mL of extract filterate, 1.5 mL of 1% HCl was added. The solution was then heated in water bath, 6 drops of Mayor's reagents/Wagner's reagent/Dragendroff reagent were added. Formation of orange precipitate was observed to show the presence of alkaloids.

Saponins. This was done according to Sofowora [11]. In brief, 2 g powder of extract was dissolved in 3ml of water and solution was shaken vigorously. A stable persistent froth was observed few drops of Olive oil was added to the froth and shaken vigorously after which an emulsion was observed.

Cardiac Glycosides. This was investigated according to Trease and Evans [12], briefly 1 mL glacial acetic acid and 2 drops of $FeCl_3$ were added to 2 mL ethanolic filtrate of extract, followed by 1 mL of concentrated H_2SO_4. A brown ring at the interface was observed indicating availability of cardenolides. A possible violet ring may appear just below the brown ring, whearas in the acetic acid layer a greenish ring may form above the brown ring and spread gradually throughout the layer.

Terpenoids. The method of Harborne [13] was implored in this analysis. Briefly 5 mL $CHCl_3$, 2 mL acetic anhydride, and concentrated H_2SO_4 were added carefully to 2 mL of the aqueous extract to form layer. Reddish brown coloration at interface was observed.

Flavonoids. According to Parekh and Chanda [9], Few drops of concentrated HCl was added to 2 mL filtrate of extract, followed by addition of 0.5 g of zinc or magnesium turnings. After 3 minutes, the appearance of magenta red or pink colour after confirmed flavonoids

Phenolics. This was executed according to Trease and Evans [12] where 1 mL of 1% ferric chloride solution was added to 2 mL of the aqueous extract, a blue or green colour indicated presence of phenols.

Phlobatannins. According to Trease and Evans [12], 2ml of the extract was boiled with 1% aqueous hydrochloride. Observation of a red precipitate inferred phlobatannins.

Steroids. This was done according to Mbatchou and Kosoono [14], where 2ml of the extract was dissolved in 10ml of chloroform followed by addition of 10ml of concentrated sulphuric acid by the side of the test tube. The upper layer turned red while, the sulphuric acid layer turned yellow showing green fluorescence. This indicates the presence of steroids.

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Phenol content. The total phenol content of R.V extracts was determined using the standard method [15]. Briefly, adequate dilution of RV extracts were oxidized using 2.5mL of 10% Folin Ciocalteaus reagent (v/v) and neutralized by 2.0mL of 7.5% sodium carbonate. The reaction mixture was then incubated for 40min at 45^{0}C and measured at 765nm absorbance in the spectrophotometer. The total phenol content was then calculated using tannic acid as standard.

Pysiochemical analysis

Proximate Analyses

This analysis was determined according to AOAC [16]. The Parameters investigated included moisture (930.04), ash (930.05), fat (2003.06), and crude fibre (962.09).

Determination of Heavy Metal

Atomic Absorption Spectrophotometer (AAS) was used to estimate the following metals: Cadmium (Cd), Manganese (Mn), iren (Fe), Zinc (Zn), Chromium (Cr) and Nickel (Ni) according to Oloyede *et al*. [17]. 2 g of sample was placed in crucible and heated at 550°C in muffle furnace for 6 h. The resultant ash was then placed in a container containing 5ml of concentrated nitric acid for digestion. It was then evaporated utilising hot plate. Small amount of distilled water was then added to the digested residue, filtered and volume was made to 30 ml using distilled water. AAS was then used in determining the quantitative analysis of the solutions produced.

Statistical analysis

Statistical analyses were performed using Microsoft Excel. Data expressed as Mean ± SEM.

Results

Phytochemical analysis

The pharmacognostic screening confirmed the absence of tannin and anthraquinone, but presence of other phytochemicals in varying proportions. Cardiac glycosides, steroids and flavonoids were more prevalent followed by alkaloids, steroids and terpenoids as presented in table 1.

Table 1: Quantitative analysis of phytochemicals present in ethanoic extract of *Rauwolfia vomitoria* dry leaves.

Phytochemicals	Inference	mg/100g
Cardiac glycoside	+++	30.15 ± 2.21
Flavonoid	+++	21.26 ± 0.73
Alkaloid	++	13.90 ± 0.27
Phenol	+++	27.30 ± 0.12
Saponin	++	13.47 ± 0.72
Steroid	+++	28.10 ± 0.17
Terpenoid	+	7.75 ± 0.21
Tannin	-	0.00
Anthraquinone	-	0.00

Key: (+) = indicate present; (-) = indicate absent
Values are duplicates expressed as Mean ± SEM

Proximate analysis

The proximate analysis of *Rauwolfia vomitoria* as shown in table 2 below revealed that moisture content had the highest concentration, followed by crude fibre. Ash content was extremely insignificant, while fat was not detected.

Table 2: Proximate analysis result of *Rauwolfia vomitoria.*

Proximate contents	Dry leaves of Rauwolfia vomitoria %
Moisture	6.40
Crude Fibre	4.25
Ash Content	<0.001
Fat Content	0.0

Mineral element composition (HEAVY METALS)

From the heavy metal screening (table 3) iron was more prevalent in the plant (540.81mg/kg) followed by manganese (31.04mg/kg), zinc was observed to be 29.69mg/kg, chromium; 24.22mg/kg, copper; 8.16mg/kg, nitrogen; 0.35mg/kg. Lead, nickel and cadmium had the same concentration of <0.001 ranking the lowest among the heavy metals

Table 3: Result of heavy metals of Rauwolfia vomitoria (Serpent Wood).

Heavy metals	Dry leaves of *Rauwolfia vomitoria* mg/kg
Nitrogen	0.35
Iron	540.81
Lead	<0.001
Copper	8.16
Nickel	<0.001
Cadmium	<0.001

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Zinc	29.69
Chromium	24.22
Manganese	31.04

Discussion

One major method for determining the quantity and quality of minerals availability in plants is moisture determination, whereby the humidity levels in medicinal plants does not attain the recommended level of 17% [18, 19]. In this study, proximate analysis of the leaves of RV revealed the presence of high amount of moisture (6.40%), followed by crude fibre (4.25%), others such as Ash was faintly detected at <0.001%) while fat was not detected. The presence of high percentage of crude fibre suggest that the leaves could be used in managing diseases such as cancer, diabetes and cardiovascular diseases [20]. However high caution should be applied when administering plants with high crude fibre or adding it as supplements in diets of infants and weaning children, as high fibre can stimulate gut mucosaa irritation in infants [20].

Macro and micro elements play critical roles in living organisms such as maintenance of some physicochemical activities in relation to lipoproteins, nucleoproteins, chromoproteins and metalloproteins [21]. The elemental screening and quantification of the leaves of RV revealed the significant presence of Fe, Mn, Zn, Cr, Cu, and N as the major and trace elements detected. The concentration of major and trace elements were observed to be in decreasing order of Fe>Mn>Zn>Cr>Cu>N while Cd, Ni and Pb were found to be present at <0.001 level beyond instrument's detection. The availability of these minerals in fluids and tissues of the body helps in facilitating the normal metabolic and physiologic processes [22, 23, 24]. Metals like Mn, Zn and Cu found in RV have been described as acting as antioxidants [25] thereby suggesting that RV might possess antioxidant potential, hence giving credence to the folkloric claims of its medicinal role. Metals like Cd and Pb that were not detected in RV suggest the non-toxicity of the plant as such metals have reported to be toxic to human body [26]. The distribution of the elements in the leaves was subjected to Dietary Reference Intake (DRIs) which is a general term for nutrients reference values comprising Recommended Dietary Allowance (RDA), Tolerable Upper Intake Level (UL), Estimated Average Requirement (EAR) and Adequate Intake (AI) which refers to the highest amount of daily mineral intake that will not be harmful to health of human (Trumbo et al., 2002) The consumption of the plant leaves can be regarded as safe as the mineral values are within Tolerable Upper Intake Level(UL). Fe is very essential in the circulatory system for transportation of oxygen and also vital for oxidative metabolism [20]. The Recommended Daily Allowance (RDA) and Upper Intake Levels (UL) for Ni has not been fixed [27]. Cadmium and lead are reported to be the standard accepted toxic metals for human studies [28]. Mercury, cadmium and Lead have no known or defined biological function [29]. However, the Bio-accumulation of Cadmium in the liver, spleen and kidneys causes disorders [30]. In raw plant materials, the World Health Organisation (WHO) determined the highest acceptable level of Cadmium as $0.30\ mg\ Kg^{-1}$ [31]. This indicates that the Cadmium concentration in the leaves (<0.001) is grossly below the limit. In raw plant materials, the WHO affirms the highest acceptable level of Lead is $10.0\ mg\ Kg^{-1}$ [31]. This implies that the Pd concentration in RV leaves (<0.001) is below the limit, which indicates that RV leaves can safe for consumption.

Qualitative evaluations such as preliminary phytochemical screening are widely used in the discovery of phytometabolites, thereby, enabling prediction of pharmacological operation of plants (Jeevitha et al 2021). The presence of alkaloids flavonoids, cardiac glycosides, steroids, phenols and terpenoids elucidates the medicinal potential of RV. Alkaloids are biproducts derived from aminoacids [32]. They are analgesic, anti-puretic and stimulate the central nervous system. Flavonoids have been reportedly abundant in plants, possessing benzoyl-y-pyrone structure [33]. Flavonoids are produced by phenylpropanoid pathway and they consist of an enormous group of compounds of polyphenol [33]. Reports has indicated that metabolites such as flavonoids are responsible for numerous pharmacological activities exhibited by medicinal plants [34, 6]. This flavonoids availability may indicate that the extract capability to alleviate cardiovascular diseases and oxidative stress since flavonoids are established potent biological antioxidants [35]. Navarrete [36] explained that tannin refers to complex large biomolecule having polyphenol nature with sufficient hydroxyls and other groups like carboxyl and possessing the ability to form strong complexes with several macromolecules. Tannins partake in healing during inflammation, burn, piles, and gonorrhea [37]. Secondary metabolites such as saponins are very important and ubiquitous in the plant kingdom, they are abundant in herbs, legumes and vegetables [38, 39]. They have also been detected in highly medicinal plants such as vernonia amygdalina, persea Americana, rhamnus prinoides and mangifera indica [33], its presence in RV suggest the pharmacologic potency of the plant. The presence of steroids may suggest that RV contain agents that can be anti-inflammatory, promote the synthesis of sex hormones [40]. Terpenoids which are molecular synthesis produced by plants have been reported to exhibit significant pharmacologic activities against malaria, cancer, viruses, bacteria and inflammation [37], it can therefore be assumed that RV may

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possess agents against such health malaise as cancer, viruses and bacteria. Derong et al. [41] described Phenols as compounds synthesized in the shikimic acid of plants and pentose phosphate via phenylpropanoid metabolization.. Saponins have the ability to form complexes with bile acids and cholesterol, thereby preventing their absorption through small intestine, hence aids in lowering the blood and liver cholesterol level [42], they function as antioxidants thereby preventing DNA degeneration [43], saponins have been taken orally and also implicated as adjutants in vaccines in the management of retroviral diseases. saponins have been reported to induce lymphocyte response against infections, stimulate synthesis of antibody and checkmate viruses [43]. Therefore the presence of saponin in the plant may confirm it as an effective buster of immunity.

Conclusion

The findings from the proximate investigation, elemental composition and pharmacognostic screening suggest that the leaves of RV is devoid of toxicosis, and due the presence of certain phytochemicals, can be of immense pivotal essence in the discovery of drugs. The outcome of this study therefore calls for the quest for further phamarcognostic investigation giving priority to those phytochemicals whose activities have been implicated in the management of cancer.

Conflict of Interest

I declare that there is no conflict of interest.

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