



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

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PRELIMINARY STUDY OF *RAUVOLFIA MEDIA* EXTRACTS AND THEIR ANTIOXIDANT ACTIVITY

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Received 16th July 2022; Revised 20th Sept. 2022; Accepted 23rd Jan. 2023; Available online 1st Oct. 2023

<https://doi.org/10.31032/IJBPAS/2023/12.10.7445>

ABSTRACT

Medicinal plants hold a very important place in everyday life. By their bio-availability, the population tends to use them to treat different diseases. The aim of this research is to know the different phytochemical compounds found in the different extracts of foliage and bark of *Rauvolfia media* followed by a quantitative estimation of the major phytoconstituent present in the extracts and the evaluation of the antioxidant activity. The present investigation includes a phytochemical screening of the barks and leaves extracts, followed by a quantitative analysis of the extracts where the total flavonoid and phenolic content of *R. media* part were evaluated by aluminum chloride and Folin-Ciocalteu respectively, and finally the evaluation of the scavenging effect by 1,1-diphenyl, 2-picryl hydrazyl (DPPH) assay. The screening results showed that alkaloids, saponins, tannins, phenols, and flavonoids are present in all fractions. The quantitative analysis conveyed that the highest concentration of phenols was found in the barks with 5.208 ± 0.15 mg GAE/g, while the highest levels of flavonoids in the leaves with 15.868 ± 0.05 mg QE/g. The methanolic fraction of the leaves possessed the highest percentage scavenging activity with an IC₅₀ of 1.038 μ g/ml compared with ascorbic acid, used as standard (1.057 μ g/ml), at a concentration of 120 μ g/ml.

Keywords: *Rauvolfia media*, screening, Phytochemical screening, Antioxidant activity

1. INTRODUCTION

Humans and animals have been gifted by an abundant presence of plants and trees with pharmacological properties (anti-microbial, anti-inflammatory, anti-analgesics, anti-helminthic, anti-hemolytic), which help to maintain a healthy life. The last few decades, researchers and scientist curiosity have increased to study the strong significance of medicinal plants and their role in order to prove their use in traditional medicine. Considering the progress of research and the advancement in technology carried out using these medicinal plants, led to the discovery of novel drugs from the bioactive compounds against diseases as cancer, AIDS, tuberculosis, diabetes, atherosclerosis, chronic inflammatory and neurodegenerative diseases, so on [1].

One of the essential factors leading in the pathogenesis of countless of these diseases is oxidative stress, caused by other reactive oxygen species (ROS) responsible for the human aging [2] and the free radicals. In all aerobic existence, the alteration of oxygen to free radicals like Oxygen free radical ($O^{\cdot-}$), Hydroxyl ion (OH^{\cdot}), and Hydrogen peroxide (H_2O_2), happens as a component of usual metabolism. In a healthy person, there is a balance between ROS generation and elimination. Free radicals

might be whether harmful or helpful to the body.

Where there is an imbalance in the formation and removal of free radicals, antioxidants act by delaying or inhibiting oxidative damage [3]. The key characteristic of an antioxidant is its ability to trap free radicals. They can be classified following their mechanisms of action: reducing activity, free radical scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen. Antioxidant compounds like phenolic acids, polyphenols, triterpenoids, steroids, steroidal glycosides, alkaloids, and flavonoids have been reported to scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the propagation of oxidative mechanisms that lead to degenerative diseases [4-6]. Researchers have demonstrated that flavonoids have been reported to be associated with antioxidative action in biological systems, performing as scavengers of singlet oxygen and free radicals [7, 8].

The genus *Rauvolfia*, which belongs to the dogbane family (Apocynaceae), is one of the largest family, grouping 200 genus [9]. Different monomeric indole alkaloids such as reserpiline has been found in the barks and

roots, which possess sympatholytic and hypotensive properties, with any visible depressant effects on the central nervous system. Compared with reserpine, which is present in other *Rauvolfia* spp., reserpiline does not cause gastric ulcers, has no laxative effects and lacks other side effects [10].

The antioxidant activity of the plant's crude extracts has never been tested. As a result, this research is the first to identify *R. media*'s considerable *in vitro* antioxidant properties. The objective of the current study is to screen the major groups, determine the total phenolic and flavonoid content of the aforementioned plants as well as their antioxidant activity.

2. MATERIALS AND METHODS

2.1. Plant samples collection

Rauvolfia media bark and leaves were harvested in Mahajanga Madagascar. Identified and certified by an herbalist of medicinal plants and by a specialist in Ethnobotany, Ecology and Conservation at the University Of Mahajanga Madagascar, EDEN Graduate School Of Natural Ecosystem.

2.2. Plant extraction

The preparation of *Rauvolfia media* extracts were carried out with different solvent. The barks and leaves (1 gm each) were ground into a fine powder. Then 1g of

the leaves (L) and barks (B) powder were extracted in 100 ml of acetone (Ac), acetic acid (Ac. Acid), methanol (MetOH) and distilled water (H₂O) by soxhlet at a different temperature. The solvents were then evaporated and concentrated to dryness using a rotary evaporator at different temperatures to obtain dry extracts and used for further study [11].

Those extracts were weighed to calculate the yield by the formula below:

$$\text{Yield (\%)} = \left(\frac{\text{Mass extract}}{\text{Mass powder}} \right) \times 100$$

2.3. Phytochemical screening

The phytochemical screening were carried out on the plant material parts, for identification of the major natural chemical groups, by using the following standard methods for alkaloids, flavonoids, tannins and phenolic compounds, saponins, terpenoids, amino acids and proteins, carbohydrates, glycosides, steroids. General reactions revealed the presence or absence of the compounds in the extracts tested.

2.3.1. Test for alkaloids

2ml aliquot of plant extracts were treated with the Dragendorff's reagent. The immediate production of an orange red precipitate indicates the presence of alkaloids [12].

2.3.2. Test for flavonoids

1ml of the extracts were mixed with one ml of ferric chloride. The presence of flavonoids were confirmed by the formation of brown color [12].

2.3.3. Test for tannins and phenolic compounds

1ml of barks and leaves extracts were treated with few ml of 5% neutral ferric chloride. The presence of tannins was shown by the presence of dark blue or bluish black color product [13].

2.3.4. Test for saponins

About 1ml of *R. media* extracts were diluted separately with 20ml of distilled water and shaken in a graduated cylinder for 15 minutes. A 1cm layer of foam indicates the presence of saponins [13].

2.3.5. Test for terpenoids

Libermann test: to 1ml of extracts, 3 ml of acetic acid and few drops of concentrated sulphuric acid were added. The color change from red to blue indicate the presence of terpenoids [14].

2.3.6. Test for amino acids and proteins

Rauvolfia media extracts were treated with one ml of 40% sodium hydroxide solution and two drops of 1% copper sulphate reagent. A violet color signify the presence of proteins [14].

2.3.7. Test for carbohydrates

Fehling's test: the extracts were mixed with 5ml of fehling's solution (A and B) and kept at boiling water bath for 5 min, which resulted by the formation of yellow or red color precipitate indicating the presence of reducing sugar [15].

2.3.8. Test for glycosides

For glycosides identification, to the extracts in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid were added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer which indicates the presence of glycosides [16].

2.3.9. Test for steroids

Libermann-Burchards test: to 1ml plant extracts, 1ml of concentrated sulphuric acid was added followed by the addition of 2ml of acetic anhydride solution. A greenish colour developed to blue indicates the presence of steroids [16].

2.4. Quantitative analysis

2.4.1. Estimation of total flavonoid content

The total flavonoid content of *R. media* barks and leaves extracts were determined by using aluminium chloride method [17] with some modifications. 0.5ml of sample (1mg/ml) was mixed with 1ml of 10% aluminium chloride, 1ml of potassium acetate (1M) and 2.5ml of

distilled water. Quercetin is used to make the calibration curve. The absorbance of all mixtures are measured using a UV-spectrophotometer at 415 nm. The total flavonoid content is expressed in terms of quercetin equivalent (mg QE/g of Sample). All the analyses is repeated three times and the mean value of absorbance was obtained.

2.4.2. Estimation of total phenolic content

The total phenolic content was determined using a spectroscopic method with a slight modification [18]. The reaction mixture was prepared by mixing 1 ml plant extracts (1mg/ml), 1ml of 10% Folin-Ciocalteu's reagent dissolved in 13ml of distilled water followed by the addition of 5ml of 7% Na₂CO₃ solution. The mixture was mixed and kept in the dark at room temperature for 2h. The absorbance was recorded using a spectrophotometer at 760 nm. All the analysis was done in triplicate and the mean value of absorbance was obtained. The TPC was expressed as gallic acid equivalent (mg GAE) per gram of the dried sample.

2.5. DPPH free scavenging activity

The free radical scavenging activity of the fractions was measured in vitro by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) [19, 20] with some modifications. The stock solution was prepared by dissolving 24 mg

DPPH with 100 ml methanol and stored at 20°C until required. A 3 ml aliquot of this solution was mixed with 100 µl of the sample at various concentrations (20 - 120 µg/ml). The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. A discoloration observed in the samples from purple to yellow and pale pink were considered as strong and weak positive respectively. Then the absorbance was taken at 517nm.

The control was prepared as above without any sample. The percent inhibition was calculated from the following equation:

$$\% \text{ Scavenging effect} = [(Ac - As)/Ac] \times 100$$

Where Ac: absorbance of control and As: absorbance of sample

The IC₅₀ values (µg/ml) were estimated from concentration vs. antioxidant activity graphs.

2.6. STATISTICAL ANALYSIS

Data are expressed as mean ± standard deviation. The IC₅₀ values of different extracts were statistically analyzed on R studio software using One-way ANOVA test followed by Tukey's HSD post hoc test (p<0.05), which is considered as significant.

3. RESULTS AND DISCUSSION

The percentages yield of both the plant extracts are summarized in the Table

1. The highest yield was recorded in the leaves extract, where the aqueous extract showed the highest percentage with 39.74%

per 120g followed by the acetone extract with 35%, when the acetic acid barks extract gave the lowest with 3.31%.

Table 1: The percentage yield of all the extracts of *R. media*

	Mass extract (g)		Yield (%) (w/w)	
	Barks	Leaves	Barks	Leaves
Acetone	5.05g	42g	4.21%	35%
Acetic acid	3.97g	10.73g	3.31%	8.94%
Methanol	12.11g	10g	10.09%	8.33%
Aqueous	11.89g	47.69g	9.91%	39.74%

3.2. Results of the phytochemical screening

Phytochemical analysis conducted on the plant extracts determined the existence of chemical constituents which are known to possess medicinal as well as physiological activities. The phytochemical screening of *R. media* extracts, shown in **Table 2**,

demonstrated that alkaloids, tannins, saponins, and flavonoids are the major secondary metabolite present in all barks fractions, while the alkaloids, saponins and steroids are mostly present in acetonic, acetic acid, methanolic and aqueous extract of barks and leave extracts.

Table 2: Phytochemical Analysis of *R. media*

	Barks				Leaves			
	Ac	Ac. acid	MetOH	H ₂ O	Ac	Ac. acid	MetOH	H ₂ O
Alkaloids	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	-	-	+	+
Saponins	+	+	+	+	+	+	+	+
Steroids	-	-	+	-	+	+	+	-
Triterpens	+	+	-	+	-	-	-	+
Glycosides	-	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	-	-	+
Phenols	-	-	+	+	+	-	+	+
Reducing sugar	-	-	-	+	-	+	-	+

(+) presence, (-) : absence

3.3. Total flavonoid content

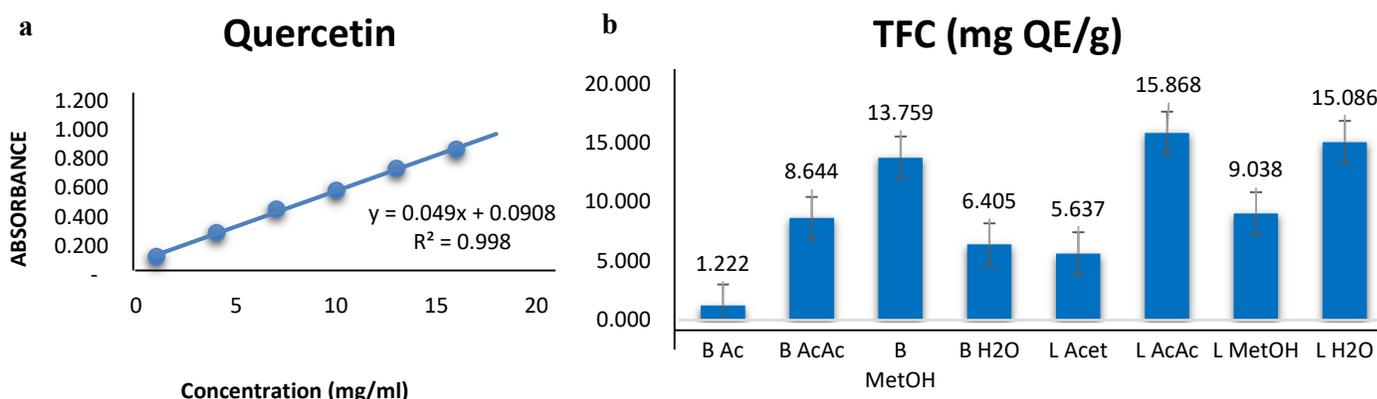
The total flavonoid content was expressed as mg/g equivalent of quercetin using a standard curve, as mentioned in **Figure 1a** with an equation: $y = 0.049x + 0.0908$ ($R^2 = 0.998$)

While the total phenolic content was evaluated as mg/g equivalent of gallic acid (**Figure 2a**) by a linear regression equation: $y = 0.0554x + 0.0885$ ($R^2 = 0.9978$)

The TFC as recorded in *Rauwolfia media* is alternated between 1.222 ± 0.10

mg/g and 13.759 ± 0.12 mg/g dry weight sample in the bark fractions, while the leaves showed a range from 15.868 ± 0.05 mg/g to 9.038 ± 0.1 mg/g. The acetic acid fraction of the leaves present the highest total

flavonoid content with 15.868 ± 0.05 mg QE/g compared to the barks with 13.759 ± 0.124 mg QE/g was found in the methanolic fraction (**Figure 1b**).



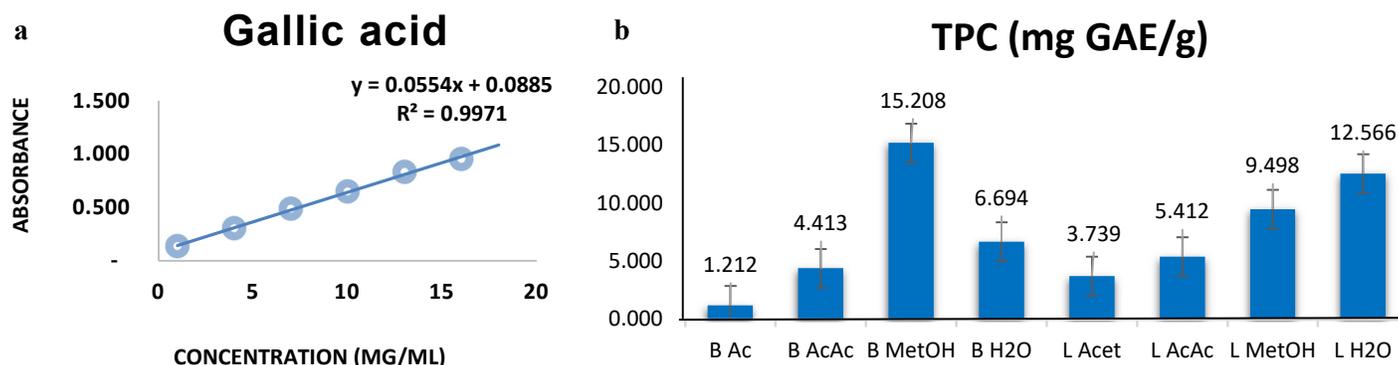
a Standard curve of quercetin **b** determination of total flavonoid content of *Rauvolfia media* barks (B) and leaves (L) extracts expressed in terms of mg QE/g. All experiments were performed in triplicate. Values are mean \pm SD (n = 3, p < 0.05)

Figure 1

Some studies demonstrated that flavonoid manifests an outstanding potential to decrease neurodegenerative disorders, risk of heart diseases and possess anticancer properties [21].

3.4. Total phenolic content

The highest TPC is found in the methanolic extract of the barks with 15.208 ± 0.15 mg GAE/g followed by the aqueous fraction of the leaves with 12.566 ± 0.257 mg GAE/g, as shown in the **Figure 2a**.



a Standard curve of gallic acid b determination of total phenol content of *Rauvolfia media* barks (B) and leaves (L) extracts expressed in terms of mg GAE/g. All experiments were performed in triplicate. Data expressed as mean \pm SD (n = 3, p < 0.05).

Figure 2

Phenolic compounds are the most imperative secondary metabolites in plants and proved to be excellent sources of accepted antioxidants, anticancer, antiatherosclerotic, antibacterial, antiviral, and anti-inflammatory activities in humans [22]. These natural antioxidants are capable of scavenging liberated superoxide radicals, protecting biological systems against the destructive effects of oxidative progressions on carbohydrates, proteins, lipids and DNA.

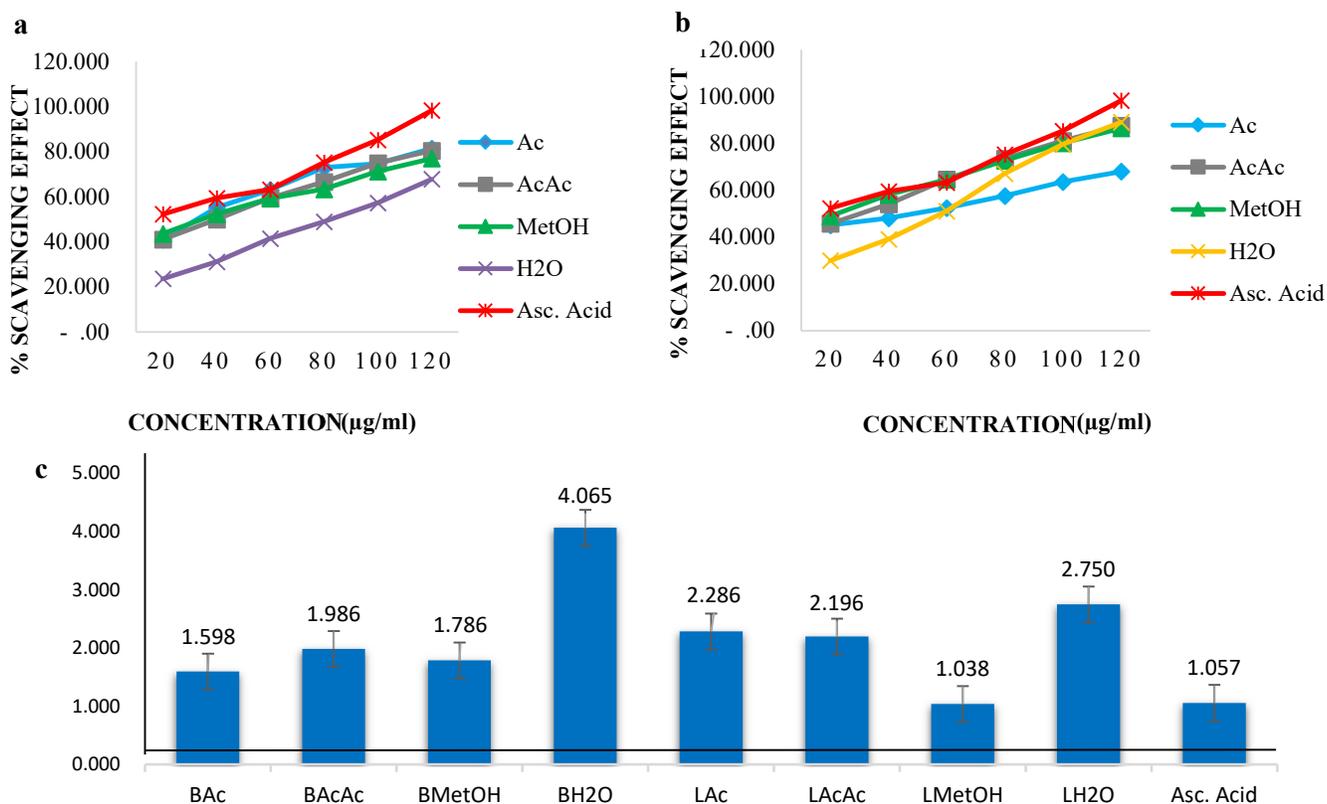
3.5. Free radical scavenging activity of *R. media* barks and leaves

Figures 3a and 3b shows the free radical scavenging activity of the different fractions of *R. media* extracts and the standard ascorbic acid. The highest activity was recorded at 120 μ g/ml, with a percentage of

81.481 \pm 0.2%, 80.463 \pm 0.09%, 7.037 \pm 0.05% and 67.840 \pm 0.15%, respectively, for Ac, AcAc, MetOH and H₂O fraction of the barks extracts, while the leaves extracts recorded 67.963 \pm 0.11% for Ac, 87.593 \pm 0.5% for AcAc, 86.481 \pm 0.01% for MetOH and 89.074 \pm 0.2% of scavenging effect for the H₂O fractions, compared to the ascorbic acid used as standard (98.33 \pm 0.24%). Among the extracts, methanolic fraction of the leaves possessed the highest activity with a lower IC₅₀ (Figure 3c) of 1.038 μ g/ml compared with the standard (1.057 μ g/ml).

The free radical scavenging activity of different extracts compared to the ascorbic acid was in the following order:

LMetOH > Asc. acid > BAc > BMetOH > BAcAc > LAcAc > LAc > LH₂O > BH₂O.



Determination of a DPPH radical scavenging activity of the barks b DPPH radical scavenging activity of the barks c IC50 of *Rauvolfia media* extracts. All experiments were performed in triplicate. Data are expressed as mean ± SD (n = 3, p < 0.05) for all tested dosages

Figure 3

Antioxidants are known to be protect cells from the injuries caused by unbalanced particles known as ROS (reactive oxygen species) and free radicals. Recent research have verified that increased formation of free radicals or reactive oxygen species (ROS) contribute to cardiovascular disease progression [23]. High antioxidant activity is reported from more than a few medicinal plants [24, 25, 26]. Researchers observed that antioxidant property in plants materials may be due to phenolics and flavonoids

compounds which are conferred in it [27, 28]. *Rauvolfia* species have received considerable interest due to the fact of their potential antioxidant activities [29]. Flavonoid possess a wide spectrum of chemical and biological activities including radical scavenging properties [30]. Antioxidant activity depends upon the number and positions of hydroxyl groups, other substituents and glycosylation of flavonoid molecules [31]. With this study, the results showed that each extract of *R.*

media barks and leaves demonstrated significant scavenging property on DPPH. Thus, concluded from the study that the plant extracts can be considered as an antioxidant and can may be used for the management of different diseases caused by oxidative stress.

4. CONCLUSION

The results from the present study on *Rauvolfia media* showed that the leaves and bark extracts have antioxidant activity. The effect might be caused by the presence and interaction between the phytochemical compounds in the plants as the preliminary screening demonstrated. All of these findings demonstrate and support the tribal population's long-standing use of plant parts, demonstrating the validity of traditional medicine as a source of information for the creation of novel drugs. The isolation, identification, and characterization of the molecule in charge of the activity require additional research and study.

Conflicts of interest

No conflicts of interest

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