

Phyto- Constituents and Antioxidant Activity of *Rhus abyssinica*, *Heeria insignis* and *Lannea schimperi* (Anacardiaceae family)

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Abstract:

For centuries, plants have been used in traditional medicines and there has been recent interest in the chemopreventive properties of compounds derived from plants. In the present study, methanolic extract of Rhus abyssinica, Heeria insignis and Lannea schimperi leaves, stem and roots were evaluated by employing in vitro anti-oxidant using 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) scavenging. Quantitative analysis of the different parts of Rhus abyssinica, Heeria insignis and Lannea schimperi for phenolic, flavonoids, alkaloids and tannins compounds revealed that the total phenolic content ranged from 79.47 to 292.79 mg/g of dry weight of leaves of H. insignis and L. schimperi extracts respectively which

expressed as gallic acid equivalents. The total flavanoids content of extracts determined by Aluminum chloride colorimetric assay ranged from the 19.24 to 43.90 mg/g of dry weight of leaves of *H. insignis* and stem of *L. schimperi* extracts respectively expressed as quercetin equivalents. The total tannins concentrations varied from 0.41 to 2.26 mg/g for *L. schimperi* stem and roots respectively. While alkaloid which has been indicated only in *R. abyssinica* its concentration ranged between 0.91 to 4.33 mg/g. The present study indicates that extracts of *Rhus abyssinica*, *Heeria insignis* and *Lannea schimperi* leaves, stems and roots are a significant source of compounds, such as tannins, flavonoids, phenols and coumarins, with antioxidant activities, and thus may be useful for chemoprevention.

Key words: Constituents, Antioxidant Activity, *Rhus abyssinica*, *Heeria insignis*, *Lannea schimperi* (*Anacardiaceae* family)

INTRODUCTION:

Natural products today are most likely going to continue to exist and grow to become even more valuable as sources of new drug leads. This is because the degree of chemical diversity found in the natural products is broader than from any other source, and the degree of novelty of molecular structure found in natural products is greater than that determined from any other source [1]. A wide range of medicinal plants part is used as raw drugs and they possess varied medicinal properties. The activity may reside in a variety of different components, including aldehyde and phenolic compounds, therefore, natural products research remain one of the main means of discovering bioactive compounds. Until recently, most natural products chemists have been more concerned with the isolation and structural elucidation of secondary metabolites than their biological activity. They realize that the, detection, isolation and structure determination of metabolites are only the first step toward answering much broader questions [2].

Many medicinal plants have been shown to contain large amounts of effective antioxidants, and the search for antioxidants and radical scavengers from natural sources is pursued worldwide, and is a key focus of our research group [3]. *Anacardiaceae* (the cashew or sumac family) family include 83 genera with about 860 known species, bearing fruits that are drupes and in some cases producing urushiol, an irritant. The *Anacardiaceae* include numerous genera with several of economic importance.

The active ingredients in *Anacardiaceae* family plants are mixtures of homologous long chain phenolic compounds collectively known as 'urushiol'. They are mono- or dihydroxybenzene derivatives of phenol (cardanols, catechols or resorcinols) or salicylic acid with a long alkyl or alkenyl carbon side chain [4], [5]. The phenols vary in the number and position of hydroxyl groups, in their alkyl chain length and in the number and position of double bonds in this chain. Phenolic lipids are also potentially useful in the treatment of cancer and skin diseases [6]. They can also be used as starting materials in the semisynthesis of compounds for various biological activities such as long lasting hydrophobic anti-inflammatory drugs or analogues of cannabinoids [7]. Cardanol is used in a wide range of technological applications such as in friction dusts for brake lining and clutch facings, and in polymer chemistry to form soft resins that are resistant to acids and bases. Derivatives of cardanol, cardol and anacardic acids have novel applications in dyes, pharmaceutical antioxidants and monomers for polymerization [8]. Plant species within the *Anacardiaceae* synthesize these toxic phenols to interact with their environment and ward off insect pests and microbes, to deter herbivores grazing from their leaves or to prevent seeds of other species from germinating in their proximity [9].

The main aims of this study are to evaluate antioxidant capacity (total phenolic content and free radical scavenging

ability) and to screen for phytochemicals content in leaves, stem and roots of *Rhus abyssinica*, *Heeria insignis* and *Lannea schimperi* from *Anacardiaceae* family.

MATERIALS AND METHODS

Chemicals and reagents:

Gallic acid, tannic acid, Querstin, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), sodium hydroxide, sodium nitrite, ferric chloride, potassium ferrous cyanide, sodium bicarbonate, aluminum chloride and Folin Ciocalteu reagent were bought from Sigma-Aldrich, USA

Collection and preparation

The roots, stem-bark and leaves of *L. schimperi*, *R. abyssinica* and *H. insignis* were obtained from Erkowit 700 km east of Khartoum. The plant materials were dried under shade for 14 days except the roots of *L. schimperi* which was dried for 28 days. They were ground into fine powder in preparation for extraction. The finely powdered materials were weighted separately and stored at room temperature.

General procedure of extraction

Methanol crude

A hundred g of the finely ground material was soaked in methanol solvent for 48 hours with occasional swirling to ensure thorough extraction. The soaked material were filtered and the crude extract collected in clean containers. The extract of each sample was weighed, transferred to small container and stored at room temperature until tested.

Successive extraction

A hundred g of the finely ground material was soaked in petroleum either a least polar solvent for 48 hours with occasional swirling to ensure through extraction. The soaked material were filtered and the crude extract collected in clean containers. The crude extract was dried at room temperature and weighed. The residue after extraction with petroleum either was soaked in chloroform solvent for 48 hours with occasional swirling to ensure through extraction. The soaked material were filtered and the crude extract collected in clean containers. The crude extract was dried at room temperature and weighed. The residue after extraction with chloroform was soaked in ethyl acetate solvent for 48 hours with occasional swirling. This followed by filtering, drying and weighing. Finally the residue was soaked in methanol and the above procedure was repeated.

Qualitative Tests for secondary metabolites
Phytochemical screening of the prepared extracts The prepared extracts were tested for their presence or absence of alkaloids, saponins, cardiac glycosides, flavonoids, sterols and triterpenes, sesquiterpene lactons, tannins and sugars according to methods described by Harborne, (1984) and Sofowora, (1993)

Quantitative determination of total phenols, flavonoids, alkaloids and tannins contents in methanolic extract of *R. abyssinica*, *H. insignis* and *L. schimperi* leaves, stem and roots.

1- Total Phenolics Content:

The total phenolic content of each extract was determined by adopting the method as described by Patel *et al* (2010). The total phenolic content was determined using Folin – Ciocalteu

reagents with analytical grade gallic acid as the standard and the contents were expressed as gallic acid equivalents (mg/l). A constructed curve of gallic acid with chart equation, $Y = 186.03x - 7.2774$, $R^2 = 0.996$, was used for calculating the concentrations of phenolic compounds in each extract.

2- Total flavonoids content:

The total flavonoids content was determined by adopting the method described by Patel *et al* (2010). Absorbance was measured at 510 nm against a reagent blank. Total flavonoids content was expressed as quercetin (mg/l) using the following equation based on the calibration curve $Y = 1262.5x - 211.9$, where y was the absorbance and $R = 0.997$, Total flavonoid contents were measured with the aluminum chloride colorimetric assay and expressed as percentage of quercetin equivalent per 100 g dry weight of sample.

3- Total alkaloids contents

The method described by Shamsa *et al.* (2008) was used for determining the total alkaloids. Atropine was used as standard solution, The absorbance of the complex (tested sample and Bromocresol green solution) in chloroform was measured at 470 nm against blank. The concentration of alkaloid compounds in each sample was calculated from the equation of the chart ($y = 0.2265x$, $R^2 = 0.9082$).

4- Total tannins content:

The tannins content was determined by using $FeCl_3$ and gelatin test [14]. Absorbance was measured at 510 nm against a reagent blank using Shimadzu model 1800 double beam spectrophotometer. The total tannins content was calculated using the following equation $y = 0.001x + 0.066$, where x = concentration of tannic acid (mg/l) corresponding to optical density. A calibration curve was constructed, using tannic acid

(100-800 mg/l) as standard with $R= 0.9936$ and total tannins content of the extracts (mg/l) expressed as tannic acid equivalents.

Determination of antioxidant activity

DPPH radical scavenging assay

Antioxidant activity of the extracts was estimated using 2, 2-diphenyl-1-picrylhydrazil hydrate (DPPH) *in vitro* according to the method of Villano *et al* (2007). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. Assays were performed in 96-well, microtiter plates. The absorbance was measured spectrophotometrically at 517 nm using a microtiter plate reader (Synergy HT Biotek®, logiciel GEN5). Propyl galate was used as reference antioxidant compound. Every analysis was done in triplicates.

IC₅₀ calculation:

The IC₅₀ (the concentration of test material, which possess 50% inhibition of free radicals) value was calculated from the linear regression of plots of concentration of the test sample against the mean percentage of the antioxidant activity. The IC₅₀ values obtained from the regression plots (Sigma Plots R 2001, SPSS Science) had a good coefficient of correlation, ($R^2 = 0.998$). The IC₅₀ of all the extracts and their fractions was determined by monitoring the effect of different concentrations ranging from 0.5 – 0.15 mg/ml. The IC₅₀ of extracts and their fractions were calculated using EZ-Fit Enzyme Kinetic Program (Perrella Scientific Inc, U. S. A.).

RESULTS AND DISCUSSION

Crude extract yield

Successive extraction of *Rhus abyssinica*, *Heeria insignis* and *Lannea schimperi* results indicated variation in the percentage yields. The methanol extract of studied organs, with exception of the stem of *Rhus abyssinica* gave the highest yield in form of gummy or waxy extracts, while ethyl acetate extracts recorded the lowest yield. Both petroleum ether and chloroform extracts of all organs gave relatively moderate yield. Petroleum ether extracts gave higher yields than chloroform extract except in case of leaves of *R. abyssinica* and *H. insignis* and the roots of *L. schimperi*.

Table (1): yields percentage of different extract from *R. abyssinica*, *H.insignis* and *L.schimperi*

Solvent	R.L	R.S	H.L	H.S	L.L	L.S	L.R
Petroleum ether	0.56	0.43	0.54	0.63	2.64	1.19	0.22
Chloroform	0.74	0.25	1.01	0.51	1.81	0.82	0.40
Ethyl acetate	0.34	0.11	0.07	0.10	1.11	0.05	0.17
Methanol	2.65	0.39	1.54	2.03	5.45	2.38	1.45

Phytochemical screening

In order to identify the phytochemical components in *R. abyssinica*, *H.insignis* and *L.schimperi*, chemical tests were carried out on the methanolic extracts of leaves, stems and roots. Results obtained for qualitative screening of these compounds are presented in Table (2). The major chemical classes such as phenols, flavonoids and coumarrins were detected in all extract samples and in high amounts. The roots of *L. schimperi* showed relatively low amount of secondary metabolites as compared with the others tested samples. Terpinoids present in all tested samples specially roots of *L. schimperi*. *R. abyssinica* leaves were rich in tannins. Many of these detected compounds have vital roles as mediators of

ecological interactions; that is, they have functions in ensuring a continued survival of particular organisms in often hostile environments where there is competition with other organisms [16]. From medicinal point of view the important secondary metabolites are alkaloids, flavonoids, tannins, terpenes and phenolic compounds [17]. Several chemical and biological studies have been carried out in *Anacardiaceae* family members (mainly *R. abyssinica*, *H. insignis* and *L. schimperi*) and revealed that these genera are important producer of secondary metabolites with significant biological activity such as antioxidant, ant diarrhea, antibacterial and antimalaria [18], [19].

Table (2): Phytochemical constituents of methanol extract from *Rhus abyssinica*, *Heeria insignis* and *Lannea schimperi*

Test	Reagent	R.L	R.S	H.L	H.S	LL	LS	L.R
Alkaloids	Mayer	+++	++	-	+	-	+	+
Coumarrins	KOH	++	++	+++	++	+++	++	++
Flavonoids	FeCl ₃	+++	+++	+++	+++	+++	+++	+++
Phenols	FeCl ₃	+++	+++	+++	+++	+++	+++	+++
Sterols	Lieberman	+	+++	+++	+++	++	+++	-
Tannins	FeCl ₃	+++	+	+	++	+	++	++
Terpinoids	Lieberman	++	++	+	++	++	++	+++

+++ = High concentration, ++ = Moderate concentration, + = Low concentration, - = Absent

Quantitative analysis for total phenols, flavonoids, alkaloids and tannins content in methanolic extract from leaves, stem and roots of *Rhus abyssinica*, *Heeria insignis* and *Lannea schimperi*:

The total phenolic, flavonoid, alkaloids and tannin contents of different parts of *Rhus abyssinica*, *Heeria insignis* and *Lannea schimperi* were evaluated and results are presented in Table (3). The methanol extract of the different parts of the plants samples was assayed with the sensitive Folin- Ciocalteu reagent; results were expressed as mg gallic acid equivalents per ml. A constructed curve of gallic acid with chart equation, $Y = 186.03 x - 7.2774$, $R^2 = 0.996$, was used for calculating the

concentrations of phenolic compounds in each extract. All the studied samples were rich in phenolic compounds. The amount of phenolic compounds in all plants samples was in range of (79.46999 – 292.7857) *Lannea schimperi* contained high amount of phenol content (specially the stem) compared with the other two species of studied plants. The lowest level of phenol content was detected in *Heeria insignis* stem.

The long chain phenols are characteristic of the *Anacardiaceae*. Some of these compounds have demonstrated antioxidant, antibacterial, antiplasmodial, wound healing ability and cytotoxicity [20].

The concentration of the flavonoids in each extract was determining using equation of the quercetin calibration curve ($Y=1262.5x-211.9$). The Results were expressed as quercetin equivalent per g of dry extract (mg QUE/g). All the studied samples showed moderate concentration of flavonoids. *Lannea schimperi* stem had high amount of flavonoids (43 mg QUE/g) followed by *Lannea schimperi* leaves and *Rhus abyssinica* stem (40 mg QUE/g) while *Heeria insignis* leaves possess the lowest concentration (23mg QUE/g).

Flavonoids are the largest group of polyphenolic compounds and widely distributed throughout the *Rhus* species [21], *Heeria* leaves [22] and *lannea* spieces [20].

The concentration of alkaloid compounds in each sample was calculated from the equation of the chart ($y = 0.2265x$, $R^2 = 0.9082$). Alkaloid was present only in *Rhus abyssinica* leaves and stem while others studied samples showed negative values. The range of alkaloids vary between (0.99667 - 4.3299) mg/ml for *Rhus* leaves and *Rhus* stem respectively. A previous preliminary phytochemical study on the leaves of *Heeria insignis* reveals the absence of alkaloids [22].

Tannic acid at different concentrations was used for construction of the standard curve of tannin. The standard curve was used for calculating the amount of tannins in each

extract, all studied samples had low concentrations range between (0.40891 - 2.26430 mg/ml). The highest level of tannins was determined in the roots of *Lannea schimperi*, while the lowest level was detected in the stem of the same plant. The leaves and stem of *Heeria insignis* showed the same amounts of tannins.

Table (3): Quantitative analysis of total alkaloids, flavonoids, phenolic compounds and tannins in mg/ml of methanolic extracts of *Rhus abyssinica*, *Heeria insignis* and *Lannea schimperi*.

Sample	Total phenolics	Total flavonoids	Total alkaloids	Total Tannin
R. abyssinica Leaves	168.87	19.24	0.91	1.96
R. abyssinica Stem	125.03	40.86	4.33	1.75
H. insignis Leaves	79.47	23.22	- 3.38	1.60
H. insignis Stem	151.41	38.29	- 0.41	1.61
L. schimperi Leaves	292.79	40.92	- 4.10	1.24
L. schimperi Stem	222.49	43.90	- 1.81	0.41
L. schimperi Roots	165.50	26.30	- 2.87	2.26

Total polyphenol is expressed as mg Gallic acid/g of dry plant material. Total Flavonoids is expressed as mg quercetin/g of dry plant material. Total alkaloid is expressed as mg atropine/g of dry plant material. Total tannin is expressed as mg of tannic acid/g of dry plant material.

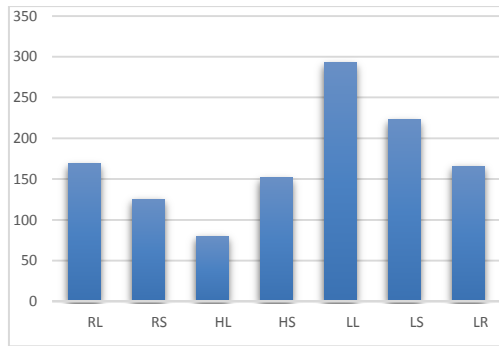


Figure (1): phenolic content in *Rush abyssinica*, *Heeria insignis* and *Lannea schimperi*

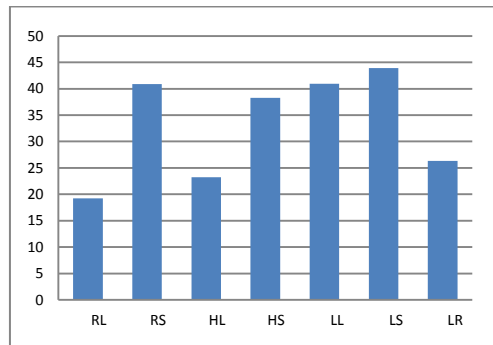


Figure (2): flavonoids content in *Rush abyssinica*, *Heeria insignis* and *Lannea schimperi*

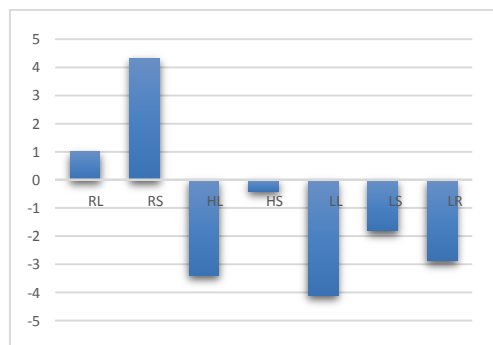


Figure (3): alkaloid content in *Rush abyssinica*, *Heeria insignis* and *Lannea schimperi*

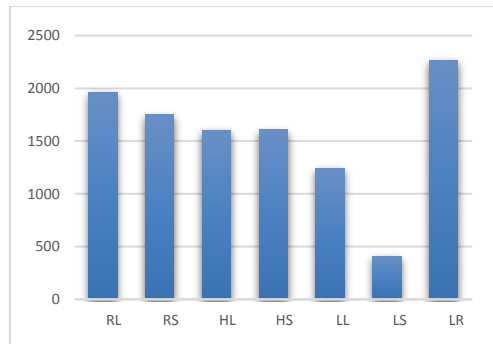


Figure (4): tannin content in *Rush abyssinica*, *Heeria insignis* and *Lannea schimperi*

Antioxidant activity:

The antioxidant activity of different plant parts from *R. abyssinica*, *H. insignis* and *L. schimperi* were determined using a solution of DPPH reagent. DPPH is a molecule containing a stable free radical. The presence of antioxidant substances could be revealed by the decrease of the intensity the purple color typical of the free DPPH radical [23]. It is a direct and dependable method for determining the radical scavenging action. DPPH is very stable free radical. Unlike *in vitro* generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally fades when antioxidant molecule quench DPPH free radicals (i.e. by providing atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them into a colourless- /bleached product (i.e. 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm band [24]. Results of antioxidant activity of crude methanolic extract were presented in Tables (4). The obtained results were compared with Propyl gallate as a good

antioxidant agent. All direct methanol crudes showed high radicals scavenging activities range between (81.9 – 92.0%) comparing with propyl gallate which showed radicals scavenging activity of 91%, the IC₅₀ value was found to be (0.1095 - 0.0475µg/ml) respectively. For successive extraction, petroleum ether extract revealed a non significant free radical scavenging activity with scavenging range between 0.3% for *R. abyssinica* stem to 7.2% for *L. schimperi* stem. All chloroform extract showed no antioxidant effect but their activities are more than those of petroleum. Concerning the antioxidant activity of ethyl acetate only three samples (*R. abyssinica* stem, *H. insignis* stem and *L. schimperi* roots) that exhibited moderate activity with scavenging rates 59.2%, 54.6% and 60.1% respectively. All methanol extract showed a high effective free radical scavenging in the DPPH assay it exhibited a remarkable antioxidant effect specially *L. schimperi* stem which showed scavenging rate of 87.2%.

Table(4): antioxidant activity of methanolic extract of *Rhus abyssinica*, *Heeria insignis* and *Lannea schimperi* using 2, 2-diphenyl-1-picrylhydrazil hydrate

Sample	% RSA ± SD (DPPH)	IC ₅₀ ± SD mg/ml (DPPH)
R. abyssinica leaves	90.8 ± 0.03	0.0707 ± 0.005
R. abyssinica stem	81.9 ± 0.03	0.1095 ± 0.002
H. insigni leaves	86.7 ± 0.01	0.0684 ± 0.008
H. insignis stem	89.7 ± 1.65	0.0268 ± 0.003
L. schimperi leaves	88.6 ± 1.55	0.035 ± 0.001
L. schimperi stem	92.0 ± 0.55	0.0475 ± 0.006
L. schimperi roots	86.0 ± 2.9	1.2129 ± 0.03
Standard	91 ± 0.02	0.0313 ± 0.02

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