PHYTOCHEMICAL INVESTIGATION AND *INVITRO* ANTIOXIDANT ACTIVITY OF AQUEOUS AND ETHANOL EXTRACTS OF *RHUS LONGIPES.*

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Abstract
The present study was undertaken to evaluate the *invitro* antioxidant activity of ethanol and aqueous extracts of *Rhuslongipes*. Ethanol and aqueous extract of *Rhuslongipes* was obtained by cold maceration and *invitro* antioxidant activity of the extracts was evaluated by using 1,1- diphenyl 2- picrylhydrazl radical (DPPH), ABTS,Superoxide radical and nitric oxide methods while the qualitative phytochemical contents was determined using standard methods.Maximum antioxidant activity was shown by ethanol extract with IC_{50} value (141.83 µg/ml) in DPPH, Superoxide (98.46µg/ml), Nitric oxide (109.23µg/ml) while the aqueous extract exhibited a better activity in ABTS with IC_{50} value (70.75µg/ml).
The result indicates that *Rhuslongipes* possesses Alkaloids, tannins, glycosides and phenolic compounds and antioxidant potential activity which could be used as a natural source of antioxidants.

Keywords
Rhuslongipes, ABTS, DPPH, Nitric Oxide and Natural Antioxidants.

Introduction

Medicinal properties of plants have been investigated in various recent scientific researches throughout the world due to their antioxidant and anti microbial activities.Antioxidants have been reported to prevent oxidative damages caused by free radicals, they interfere with oxidation process by acting as oxygen scavengers, chelating and reacting with free radicals (Shahidi and Wanasundara 1992; Buyukokuroglu , 2001).
Reactive derivatives of oxygen, known as Reactive Oxygen species (ROS) are continuously generated inside the human body, this ROS are detoxified by the antioxidants presents in the body. However, the over production of ROS or the insufficiency of antioxidants in the body leads to oxidative damages to various biomolecules such as proteins, lipids, DNA and lipoproteins (Farber, 1994).

Hence, the Pathogenesis of several diseases such as arteriosclerosis, diabetics, cancer and malariahas been attributed to oxidative stress (Alho and Leinnone 1999).
The use of plants extracts with established antioxidant properties could be of great importance in the prevention of various ailments, Several phytochemicals found in medicinal plants are known to be responsible for anti-cancer, anti-microbial, and antioxidant activity such as Alkaloids, flavonoids, tannins and quinines (Kumar et al .,2007).
Rhuslongipes or Searsialongipes (Engl) is one of the commonly used plants in the treatment of malaria, Asthma,wounds in Ilaro, Ogun state, south west Nigeria. *Rhuslongipes* have been reported according to Burkil, (2004) to be used in the treatment malaria and cancer. Due to the scanty in literature of information on the pharmacological and biological activities of the plant, *Rhuslongipes* genus has been reported to demonstrate significant pharmacological activities. These present studies therefore aim to evaluate the *invitro*antioxidant activities of aqueous and ethanol leave extracts of *Rhuslongipes.*

2. Materials and Methods

2.1 Plant Collection

The fresh leave of *Rhuslongipes* were collected in the month of January 2018 at OjaOdan, Ogun state,Nigeria. The plant was identified and confirmed by Dr P.O Bankole of Collegeof Natural Sciences, Department of Pure and Applied Botany, Federal university of Agriculture, Abeokuta. Ogun State.

2.2 Preparation of plant Extract

Ethanol extraction of the plant was carried using a method of Talent, Mohammed, Neil and Shahidul (2015). 180 g of the dried pulverized leaves were macerated in each of two 5L round bottomed flask containing 1.6L of 95% ethanol solvent in shade for 72 hours with shaking of the extracts in the intermediate time. The extract was filtered through a Whatman filter paper No. 1 and the filtrate collected was concentrated using rotary evaporator model (RE 300) which ensures evaporation of bulky solution to smaller volume concentrates (semi-solid) at temperature of 40^{o}C. The extract was weighed and transferred to micro tubes and stored in a refrigerator at 4^{o}C until required.

2.3. Phytochemical Screening

The preliminary phytochemical screening was performed on aqueous and ethanol extracts of *R. longipes* leaves using standard procedures described by Evans (1997). The extracts (5mg) were dissolved in 50ml of the respective solvents used for extraction. The solution was made ready for qualitative phytochemical analysis for Alkaloids, tannin, glycosides, phenol, saponins, carbohydrates, proteins.

2.4 ABTS(2, 2'-azino-bis 3-ethylbenzthiazoline-6- sulfonic acid) radical cation scavenging)

ABTS radical scavenging activity of *R.longipes* was determined according to the method of Re, Pellegrini, Proteggente, Pannala, Yang and Rice-Evans (1999). ABTS radicals were pregenerated by adding 5mL of a 4.9mMpotassium persulfate solution to5mL of a 14mMABTS solution and kept for 16 h in the dark. Different concentrations of extract (10–150 μg/mL) were added to the above activated pregenerated ABTS solution. This solution was suitably diluted with distilled water to yield an absorbance of 0.70 at 734 nm and then used for antioxidant assay. Ascorbic acid (50 μg/mL) was used as reference compound. 50 μL was added to 950μL of ABTS solution and vortexed for 10 s and after 6min and then reduction in absorbance was recorded at 734 nm, using distilled water as a blank, on ELICO (SL-150) UV-visible spectrophotometer (Sanathnagar, Hyderabad, Andhra Pradesh, India). Same volume of test solutions of each extract was also taken in similar manner. The result was compared with control (only ABTS solution) having absorbance 0.712 ± 0.032.

2.5 DPPH (2, 2'-diphenyl-1 -picrylhydrazyl) free radical scavenging assay

DPPH inhibition in *Rhuslongipes* was determined by using the protocol of Brand-Williams, Cuvelier and Berset (1995) with some modifications of Mensor, Menezes and Leitao (2001).The DPPH radical (Hi media) is stable due to the delocalization of a spare electron over the molecule, thus preventing dimer formation. This radical is used in the DPPH radical scavenging capacity assay to quantify the ability of antioxidants to quench the DPPH radical. The dark purple color of DPPH will be lost when it is reduced to its non-radical form stable organic nitrogen centered free radical with a dark purple color which when reduced to its non-radical form by antioxidants becomes colorless. DPPH radicals are widely used in the model system to investigate the scavenging activities of several natural compounds. When the DPPH radical is scavenged, the color of the reaction mixture changes from purple to yellow with decreasing of absorbance at wavelength 517 nm. 200mg of sample was taken in centrifuge tube (in triplicate). Two hundred microliter distilled water was taken in blank instead of the sample. Then 1mL of DPPH (8 mg/100mL of ethanol) solution was added to the sample and the blank. This setup was left at room temperature for 30 minutes (vertexed in between). Tubes were then centrifuged at 4000 rpm for 10 min. After that, 0.5mL supernatant was poured in fresh tubes containing 1mL of ethanol (ethanol absolute 99.9%, analytical reagent, ChangshuYangyuan Chemical, China) and the absorbance was taken at 517nm against the ethanol by using UV-1800 spectrophotometer (Shimadzu, Japan). Each crude extract was analyzed in triplicate. The percentage of inhibition was calculated against blank: $I\% = A$ blank $- A$ sample A blank $\times$ 100, (2) where Ablank is the absorbance of the control reaction (containing all reagents except the test compound) and Asample is the absorbance of the test compound.

2.6 Superoxide Radical Scavenging Activity

Superoxide radical scavenging potential ethanol extracts of*R.longipes*was reported in terms of its capacity to inhibit the formazan formation upon photochemical reduction of nitro bluetetrazolium (Beauchamp and Fridovich1970). In brief, each 3mL reaction mixture (0.01M phosphate buffer (PH 7.8), 130mM methionine, 60 μM riboflavin, 0.5mM EDTA, NBT (0.75mM) with 0.5mL extract/CuSO4 solution; positive Control). These tubes were kept in front of fluorescent light for 6 minutes and absorbance was taken at 560 nm. The non-enzymatic phenazine methosulfate-

nicotinamide adenine di-nucleotide (PMS-NADH) system generates superoxide radicals, which reduce NBT to a purple formazan. The decrease in absorbance at 560 nm with the plant extract and the reference compound quercetin indicates their abilities to quench superoxide radicals in the reaction mixture. Identical tubes were kept in the dark and served as blanks. The results were expressed in percent inhibition as compared to control.

2.7 Nitric oxide radical scavenging assay

Nitric oxide generated from aqueous sodium nitroprusside (SNP) solution interacts with oxygen to produce nitrite ions at physiological pH, which may be quantified and determined according to GriessIllosvoy reaction (Green, Wagner,Glogowski,Skipper,Wishnok and Tannenbaum 1982). The reaction mixture contained: 10mM SNP in 0.5M phosphate buffer (pH 7.4) and various concentrations (10-150 μg/mL) of *R.longipes*in a final volume of 3mL. After incubation for 60min at 37°C, Griess reagent (0.1% α-napthyl-ethylenediamine in water and 1% sulphanilic acid in 5% H_3PO_4 was added. The pink chromophore generated during diazotization of nitrite ions with sulfanilamide and subsequent coupling with α-napthylethylenediamine were measured spectrophotometrically at 540 nm. Ascorbic acid was used as a positive control. Nitric oxide scavenging ability (%) was calculated by using above percent inhibition (%) formula for DPPH assay.

3. Results

Table 3.1: Phytochemical tests of *Rhuslongipes*extract

S/N	CONSTITUENT	TEST	ETHANOL EXTRACT	AQUEOUS EXTRACT
1.	Alkaloids	wagner's test	Present	Present
2.	amino acid	ninhydrin test	Present	Present
3.	gums & mucilage	gums &mucilage test	Present	Present
4.	saponin	saponin test	Present	Present
5.	carbohydrate	benedict test	present	present
		molish test	present	present
6.	fixed oils &fats	spot test	Present	Present
7.	Phenoliccompound& tannins	ferric chloride test	present	absent
		lead acetate test	present	present
8.	phytosterols	alkaline test	present	present
		magnessium& HCl reduction	absent present	absent present
		libermann-burchard'stest	present	present

9.	Protein buiret test	present	absent
	million's test	present	absent
10.	Glycoside legal test	absent	absent

3.1**ABTS(2, 2'-azino-bis 3-ethylbenzthiazoline-6- sulfonic acid) radical cation scavenging**.
From table 1 below, the result of this present study indicated that, the IC_{50} of the aqueous extract (70.75 µg/ml) had a lower value when compared with the IC_{50} of ethanol extract of the plant (396.12µg/ml). However, the IC_{50} of aqueous and ethanol extract were compared with the IC_{50} of the standard drug ascorbic acid with IC_{50} value of (1825.4 µg/ml)

Table 1: IC_{50} values of *in vitro* antioxidant activities of *R.longipes* using ABTS radical scavenging activity

Concentration (µg/ml)	**Aqueous extract**	**Ethanol extract**	**Ascorbic Acid (VIT C)**
10µg/ml	16.658	71.771	81.173
20 µg/ml	24.845	81.017	83.006
50 µg/ml	50.129	80.526	83.781
100 µg/ml	71.978	83.987	84.143
150 µg/ml	76.085	84.272	84.349
IC_{50}Values (µg/ml)	70.750	396.128	1825.4

3.2 DPPH (2, 2'-diphenyl-1 - picrylhydrazyl) free radical scavenging assay)
.From table 2 below, the results from the experimental model (DPPH free radical scavenging assay) indicated that the leaves of *R.longipes*extracted using ethanol and aqueous extract possess antioxidant potential in a concentration dependent manner to varying extents. The ethanol extract possess stronger antioxidant activity with IC_{50} of (141.82 µg/ml) and aqueous extract with IC_{50} value of (390.82 µg/ml)while the standard drug (ascorbic acid) has an IC_{50} value of (103.63 µg/ml).

Table 2: IC_{50}value of in-vitro antioxidant activity of *R.longipes* using DPPH free radical scavenging assay.

Concentration (µg/ml)	**Aqueous extract A**	**Ethanol extract B**	**Ascorbic Acid VIT C**
10µg/ml	2.228	12.659	19.564
20 µg/ml	7.116	21.229	29.299
50 µg/ml	18.206	25.629	35.236
100 µg/ml	18.619	46.965	48.571
150 µg/ml	19.824	47.355	63.169
IC_{50}Values (µg/ml)	390.827	141.830	103.633

3.3 Superoxide Radical Scavenging Activity
Table 3, shows the radical scavenging ability and antioxidant properties was increased in ethanol extract with IC_{50} value of (93.46 µg/ml) and the aqueous extract has low antioxidant properties with high IC_{50} value (253.25 µg/ml). However, ascorbic acid has high antioxidant properties with IC_{50} value (55.05µg/ml).

Table 3: IC_{50} value of in-vitro antioxidant activity of *R.longipes* using superoxide radical scavenging assay.

Concentration (µg/ml)	**Aqueous extract A**	**Ethanol extract B**	**Ascorbic Acid VIT C**
10µg/ml	19.336	34.209	52.537
20 µg/ml	19.469	38.513	71.182
50 µg/ml	23.001	42.470	82.603
100 µg/ml	34.263	51.155	86.348
150 µg/ml	34.847	59.734	90.810
IC_{50}Values (µg/ml)	253.255	93.462	55.057

3.4 Nitric oxide radical scavenging assay

The present study (table 4) investigated that the ethanol extract has high antioxidant activity with IC_{50} value (109.22µg/ml) while the aqueous extract which has IC_{50} value (446.33µg/ml), while ascorbic acid which has good antioxidant activity with IC_{50} value (89.76µg/ml).

Table 4: IC_{50} value of in-vitro antioxidant activity of *R.longipes* using nitric oxide radical scavenging assay.

Concentration (µg/ml)	**Aqueous extract A**	**Ethanol extract B**	**Ascorbic Acid VIT C**
10µg/ml	27.806	36.735	36.862
20 µg/ml	29.719	41.390	44.324
50 µg/ml	31.569	44.898	46.684
100 µg/ml	34.184	48.597	50.638
150 µg/ml	34.885	54.273	57.079
IC_{50} Values (µg/ml)	446.335	109.229	89.769

4. Discussion

Crude extracts of herbs, vegetables, fruits, cereals, and other plant materials rich in phenolics and flavonoids, are increasingly being used in the food industry for their antioxidant properties and health benefits. In this study, the ethanol extract of *Rhuslongipes* exhibited various secondary metabolites such as alkaloids, steroids, phenols, glycosides, tannins which are known to be responsible for antioxidant, anticancer,antimicrobial activities of the plant(Kumar et al.,2007). TheABTS radical scavenging is a popular antioxidant assay that measures the radical scavenging nature of compounds which includes that of plant extracts(Katalinic*et al*., 2006; Pawlaki*et al*., 2010; Li *et al*., 2011; Huang *et al*., 2012)..In the present study, the ethanol extracts of *Rhuslongipes* demonstrated a strong scavenging potential when compared to aqueous extract. High phenolic content extracts shows marked scavenging of ABTS radicals indicating a relationship between phenolic content and scavenging of radicals.

DPPH is another antioxidant assay used to evaluate the antioxidant activity of herbal extracts. This method is rapid, simple and determines the capacity of plant extracts to bleach the DPPH radical. In the present study, the DPPH absorption of the leave extracts in the presence of vary concentration was monitored at wavelength of 517nm. The result revealed that the ethanol extracts of the extracts possess high scavenging potential when compared to aqueous extract. This is evidence that the extracts showed ability to donate hydrogen and hence, it could serve as free radical scavengers and as primary antioxidants (Chung et al., 2006). Thus, higher radical scavenging activity of ethanol extract may be attributed to higher amount of hydrogen donating phenolic antioxidants in ethanol extract. Same result was found by Roby et al (2003) for *Thymus vulgaris* and concluded that the antioxidant activities of plant extracts expressed as antiradical power (ARP) are affected by solvents used for extraction.
Superoxide are less toxic and can be generated in biological system during cellular respiration, In the presence of Iron, they are converted into highly reactive OH radical (Lushchak, 2014). The ethanol extracts possess a strong scavenging activity when compared to aqueous extract.

Nitric oxide is one of the important cellular signaling molecules involved in many pathological and physiological processes. Nitric oxide radical(NO*) becomes toxic after reacting with superoxide anion radical activity by reducing the generation of NO* in a concentration dependent manner. The standard drug demonstrated the highest activity;however the ethanol and aqueous extracts also exhibited appreciable antioxidant activity.
The result of this study also conform to a work according to Olorunisola*et al* (2017) that shows that the *Rhuslongipes* possesses antioxidant potential and can protect against paracetamol induced toxicity.

5. Conclusion
These results suggest that the extracts of *Rhuslongipes* possess some phytochemicals such as phenols, Alkaloids, glycosides and antioxidant properties which might be helpful in preventing the progress of various oxidative stresses. However, this plant could be use as a source of antioxidants for pharmaceutical use.

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