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Phytochemical Screening and In-VitroAntioxidant Activity of Acetonic, Methanolic and Aqueous Extracts of Sclerocary abirrea

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ABSTRACT: The powdered bark ofSclerocarya birrea (S. birrea) has been used for many centuries in Africa to treat several disorders. A decoction of its bark traditionally treats gangrenous rectitis, ulcer, rheumatism, dysentery, insect bites, burns, diarrhea, haemorrhoid, heavy menstruation, gonorrhea as well as malaria. Three crude extracts were prepared from the stem bark ofS. birreavia cold extraction withacetone methanol, and water respectively.The extractswere subjected to phytochemical screening and antioxidant studies. These investigations revealed the presence of carbohydrates, flavonoids, tannins, terpenoids, glycosides, saponins, sterols, and phenols, where alkaloids and quinones were not detected in all the extracts. Antioxidant activitiesof the stem bark extracts were determined using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). The antioxidant studies revealed that all the three extracts exhibited higher radical scavenging activity than the standard ascorbic acid. The IC_{50} values in the acetonic, methanolic and the aqueous extract of S. birrea were found to be **60.25, 64.21, and 60.44ppm** respectively-compared to the standard ascorbic acid where the $IC_{50}$was recorded to be148ppm.

Keywords:Phytochemicals, DPPH, Antioxidant, Sclerocaryabirrea

I. INTRODUCTION

Sclerocaryabirreacommonly known as marulais a middle-sized deciduous savannah tree belonging to the family anacardiaceae in the order sapindales mostly found in the Southern Africa,Madagascar, tropical and sahelian Africa (Coates, 1977).It is a medium sized tree reaching heights of between 7 and 17 m, with grey fissured bark, stout branches and pale foliage (Coates, 1977). The flowers are mainly greenish-white or reddish. The fruits are yellow, resembling a mango (Dimoet al., 2007). The rough stem-bark is flaky, with a mottled appearance due to contrasting grey and pale-brown patches. It has got a wide range of ethnomedicinal importance many of which have been scientifically proven(Dimoet al., 2007).A decoction of its bark traditionally treats gangrenous rectitis, ulcer, rheumatism, dysentery, diarrhoea, haemorrhoid, heavy menstruation, gonorrhea as well as malaria(Hall et al., 2000).Studies on S.birrea reveal the presence of a number of biological applications. According to the literature, it has got a host of pharmacological activities including; antidiabetic properties,anti-plasmodial, antibacterial, antimalarial, anti-inflammatory, antifungal, antihypertensive, anticonvulsant, hepatoprotective, and pesticidal activity (Dimoet al., 2007; Dieyeet al., 2008; Ojewole, 2003a; Van de Venter et al., 2008; Gathirwaet al., 2008; Nundkumar and Ojewole, 2002; Eloff, 2001; Masokoet al., 2008; Garbaet al., 2006; Ojewole, 2007).

S. birreahas multiple uses, the fruits are eaten fresh or fermented to make a beer, the kernels are eaten or the oil extracted, the leaves are browsed by livestock and have medicinal uses, as does the bark. The wood is carved into utilitarian items such as spoons and plates as well as decorative animal figures. Because of these multiple uses, and its significance in the landscape, several African cultures have specific beliefs and ceremonies associated with this species (Walker, 1989).Most of these phytochemical constituents are potent bioactive compounds found in plant parts which are precursors for the synthesis of useful drugs (Sofowora, 1993). These secondary metabolites are reported to have varied uses as antimicrobial and other physiological activities (Sofowara A, 1980). There is the need to carry out studies on antioxidant and antimicrobial studiesto determining other factors responsible for the therapeutic benefits of Sclerocaryabirrea. There has been much evidence in the literature on biological

and ethnomedicinal uses of S. birreahowever, there is no complete report on phytochemicals, pharmacological effects of the phytochemicals, and ethnomedicinal properties of stem bark extract ofS. birreafound in the Northern region of Nigeria, Yobe state. Therefore, within the scope of the current studies, the study focuses on evaluation of phytochemical constituents and antioxidant activity of S. birrea.

II. MATERIALS AND METHODS

Materials

Test tube, dropper, measuring cylinder, round bottom flask, condenser, heating mantle, beaker, conical flask, spatula, mortar and pestle, cuvet, tissue paper, clamp and cotton wool.

Equipment

Weighing balance (model/PA214) made by Chaus corporation, UV UNICO Spectrophotometer (modelNo.UV2150), S/N KP 12111212018 products of UNITED STATE AND INSTRUMENT INV. Vortex mixer Bibby scientific limited stone. Automatic shaker. K. HS 501. Acetone and methanol used were purchased from BDH Chemicals, Pools, England; DDPH, SIGMA-ALDRICH, INC, all other chemicals used were of analytical grade.

Chemicals

Methanol, acetone, distilled water, 2,2-diphenyl-1-picryl hydrazyl (DPPH), ascorbic acid, potassium iodide, iodine crystals, mercuric chloride and bismuth iodide.

Sample Collection and Preparation

The freshstem bark ofScelerocaryabirrea was collected fromYobe State University, Damaturu and kept under shade immediately after collection from the field. Thesamples were cut into smaller bits and dried under shade in the laboratory at room temperature. The dried samples were crushed with a mortar and pestle into coarse particles and the drying continued until a constant weight was obtained. The powdered sample was then extracted using three different solvents namely; acetone, methanol and watersuccessively.

Extraction Procedure

The air-dried stem bark (100g of the coarse powder) was extracted successively with 500mL each of acetone, methanol and water respectively via cold extraction method with the aid of automatic shaker for 24hours. The extracts were filteredanddried following evaporation of the solvents at 30-40°C. The resulting residue was placed in and an oven until completely dried. Finally, the dried extracts were kept in a refrigerator at 4°C for phytochemical analysis and antioxidant activity.

Determination of Antioxidant Activity

DPPH free radical scavenging activities ofSclerocaryabirreacrude extracts were determined according to the slightly modified procedure as described by Hatanoet al.,(1988). Acetonic, methanolic and aqueous stem bark extracts of the sample of different concentrations (1-100 ppm) were prepared and transferred to each of the 3mL, 0.004% ethanolic DPPH solution. The mixture was incubated at room temperature for 30 minutes, and the absorbance of each of the solutions was measured at 517nm using UV spectrophotometer, taking ascorbic acid as the standard. Noting the blank absorbance in each case, the % inhibition was calculated by the following equation:

$$\% \text{ Free radical scavenging activity } (\%RSA) = \frac{(A_{blank} - A_{sample})}{(A_{blank})} \times 100\%$$

Where $A_{sample}$and $A_{blank}$are absorbance of sample extracts and blank solutions.IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals (Gupta et al., 2003). So, the inhibitory concentration 50% (IC_{50}) values were determined based on the calibration curves obtained (percent inhibition vs concentration).

III. RESULTS AND DISCUSSION

In the present study, 100g of dried powdered stem bark sample wassubjected to cold extraction with 500ml of solvents of increasing polarities namely; acetone, methanol and water. The weights of the dried extracts were taken in order to determine the percentage yields of the extract by the three solvents; the results are presented in Table 1.

Table 1:The Percentage yield obtained from the extraction ofS. birreastem bark sample.

S/No	Sample weight (g)	Solvent	Weight of Extract	Yield (%)
1.	100	Methanol	12.-48	12.48
2.	100	Acetone	40.26	40.26
3.	100	Aqueous	3.08	3.08

Thethree extracts were subjected to qualitative phytochemical test. The class of secondary metabolites present were identified and the results obtained are presented in table 2.

The results in table 2 revealed the presence of tannins, terpenoids, glycosides, saponins, sterols, oxalate, flavonoids, carbohydrate and phenols in the stem bark extracts of Sclerocaryabirrea. Out of these, flavonoids, carbohydrate and oxalate were only identified in the acetone extracts. While alkaloid and quinone were absent in the three extracts.

Table2: Phytochemical screening results of the stem bark extracts of S.birrea.

S/No	Phytochemicals	Solvents		
		Methanol	**Acetone**	**Aqueous**
1.	Alkaloids	_	_	–
2.	Flavonoids	_	+	–
3.	Saponins	+	+	+
4.	Glycosides	+	+	+
5.	Terpenoids	+	-	+
6.	Tannins	+	+	+
7.	Sterols	–	+	–
8.	Phenols	+	+	+
9.	Oxalates	–	+	–
10.	Carbohydrate	–	+	–
11.	Quinones	–	–	–

Key:+ = Present, – = Not present

Absorbances of the ascorbic acid standard concentrations were measured at 517nm (table 3). The absorbances of the acetonic, methanolic and aqueous extracts of S. birrea were given in table 4. The antioxidant activities of the crude extracts were determined on the basis of their scavenging activity of stable DPPH free radical. The results were then compared with the corresponding absorbance of standard ascorbic acid of concentrations (1-500 ppm) taken at 517nm. Since IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals (Gupta et al., 2003), the IC_{50} values weremeasured from the graphs of % inhibition vs concentration (table 5).

Table 3:UV absorbance of ascorbic acid standard measured at 517nm.

S/No	Conc. (ppm)	Absorbance
1	5	0.65
2	10	0.643
3	25	0.542

4	50	0.235
5	100	0.088

Blank =0.671

Table 4: UV absorbance of Sclerocaryabirrea stem bark extracts measured at 517nm.

S/No	Conc. (ppm)	Acetone	Methanol	Aqueous
1	5	0.32	0.381	0.381
2	10	0.26	0.32	0.334
3	25	0.218	0.283	0.301
4	50	0.200	0.275	0.214
5	100	0.150	0.07	0.011

Blank = 0.393

Table 5: The IC_{50} value of Sclerocaryabirreaextracts and ascorbic acid.

S/N	Test Samples	IC_{50}(ppm)
1	Ascorbic Acid	148
2	Acetone Extract	60.25
3	Methanol Extract	64.21
4	Aqueous Extract	60.44

The IC_{50} values were calculated from the graph plotted as percentage inhibition against the concentration (µg/mL) as shown in Figure 1.

The IC_{50} value represents the concentration of the antioxidant required to quench 50% of the DPPH radical. The calculated IC_{50} values of ascorbic acid together with various stem bark extracts of S. birreawere presented in Table 5. It is very evident that all the three extracts exhibited higher free radical quenching capabilities than the standard ascorbic acid. Since a lower value of IC_{50} means that the sample has a higher antioxidant activity, thus the acetonic extract which showed an extremely low IC_{50} value of 60.25 µg/mL can be said to be a very potent antioxidant. This is followed by the aqueous extract with 60.44 µg/mL and methanolic extract with 64.21µg/mL respectively.

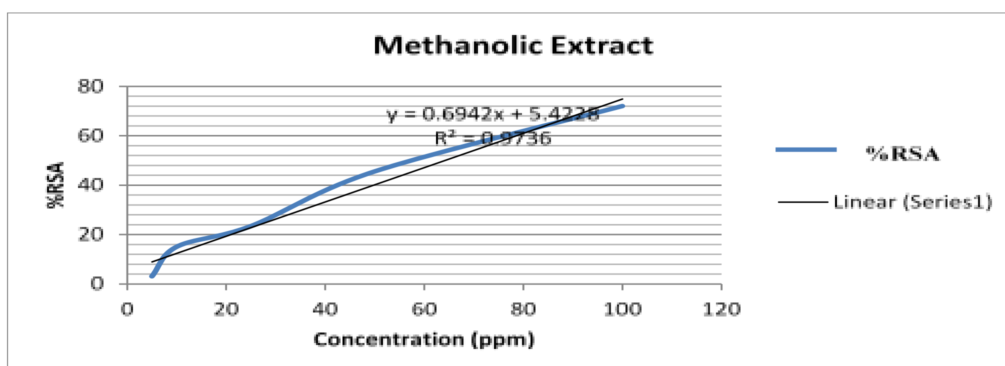

Figure 1: DPPH RSA of S. birrea Methanolic Extract

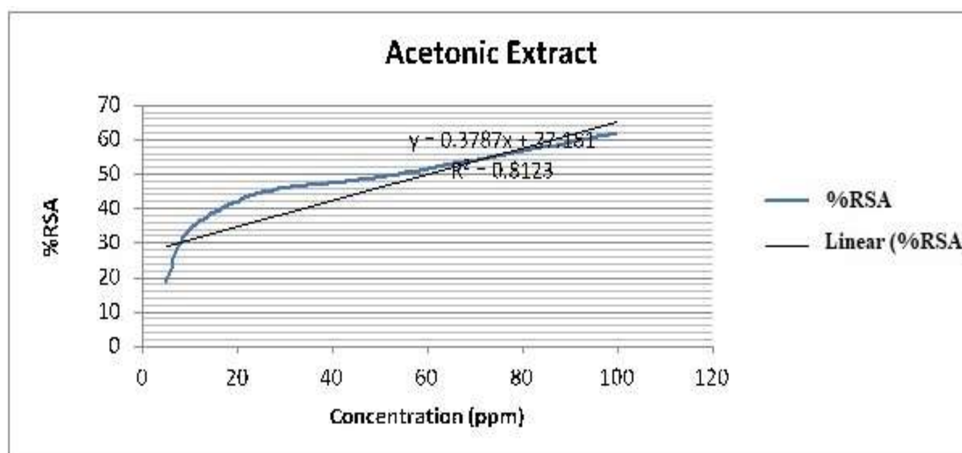

Figure 2:DPPH RSA of S. birrea Acetonic Extract

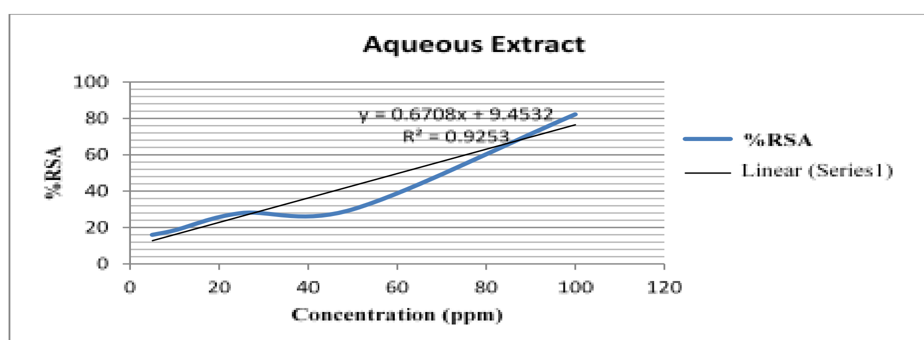

Figure 3**:** DPPH RSA of S. birrea Aqueous Extract

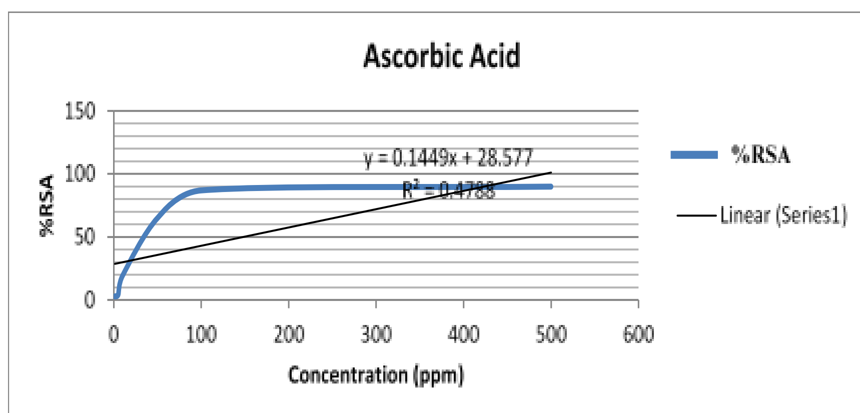

Figure 4:DPPH RSA of Ascorbic Acid

IV. CONCLUSION

The results have shown a very strong radical scavenging activity of S. birrea stem bark extracts and hence signaling pharmaceutical industries to focus on such a great plant for further pharmaceutical use. The study of the antioxidant activities of extracts and the fractions isolated from the stem bark might throw more light on how individual phytochemicals can protect cell/tissues from oxidative damages arising from reactive oxygen species and other free radicals generated during the body's metabolic processes.

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