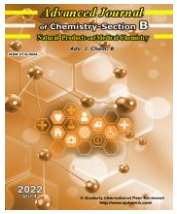
Advanced Journal of Chemistry-Section B
Natural Products and Medical Chemistry

Journal homepage: http://www.ajchem-b.com/



Original Research Article

Phytochemical Screening and Evaluation of the Antioxidant Potentials of the Stem Bark Extracts of *Erythrophleum Africanum* (African Blackwood)

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ARTICLE INFO

Article history
Submitted: 2022-06-01
Revised: 2022-07-16
Accepted: 2022-09-25
Available online: 2022-09-29
Manuscript ID: AJCB-2206-1123
DOI: 10.22034/ajcb.2022.345261.1123

KEYWORDS
Erythrophleum Africanum
Medicinal Plants
Antioxidant
Hydrogen Peroxide
Phytochem istry

ABSTRACT

Antioxidant agents have been reported to play a significant role in protecting against infections induced by oxidant damage. *Erythrophleum africanum* have been found to be a reservoir of antioxidant agents. The powdered stem bark sample was macerated with acetone, methanol, and water successively for two weeks by using standard maceration method. The mixtures were agitated with a magnetic stirrer to dissolve the particles, decanted, filtered, and concentrated on rotavapor (R110) at 40 °C to obtain the acetone, methanol, and water (aqueous) extracts. By using hydrogen peroxide (H_2O_2) scavenging assay, the antioxidant potentials of *Erythrophleum africanum* stem bark extracts was investigated for its free radical scavenging property. The preliminary phytochemical screening of the extracts revealed the presence of seven (7) different secondary metabolites including: saponins, tannins, flavonoids, quinones, glycosides, phenols, and steroids. From the H_2O_2 scavenging assay, the acetone, methanol, and water (aqueous) extracts showed good dose-dependent free radical scavenging potentials. Both acetone and methanol extracts showed remarkable antioxidant capabilities compared with the standard (ascorbic acid), with the exception of the aqueous extract, which is lower than the standard (ascorbic acid, acetone, methanol, and water: 1.851, 2.412, 2,888, and 1.289). The presence of different secondary metabolites in the stem bark extracts of *Erythrophleum africanum* proves the use of the plant in folkore medicine and confirm its use as antioxidant.

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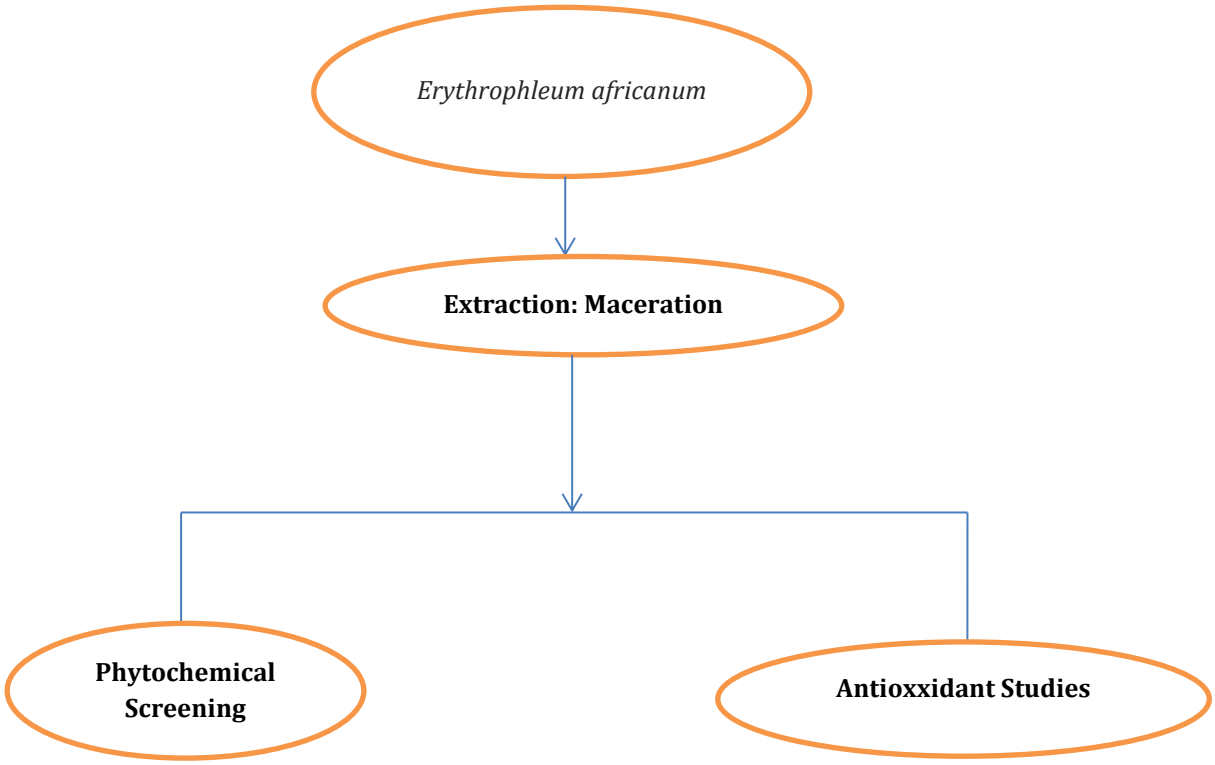
GRAPHICAL ABSTRACT



1. Introduction

Despite the tremendous progress in human medicine, infectious diseases caused by bacteria, fungi, virus, and parasites are still major threats to public health. Their impact is particularly large in developing countries due to the relative unavailability of medicine and emergence of drug resistance [1]. During the last two decades, the development of drug resistance and the appearance of undesirable side effects of certain antibiotics [2] have led to the search for new biologically active compounds from natural sources as a new antimicrobial agent with the view to discover the novel chemical structures which could overcome the above disadvantages [3]. The development of resistance to the current antibiotics by disease causing microbes has also reinforced research for the discovery of new ones. The current trends in drug development process are focused on natural sources, especially of plant origin due to some proven correlation between the folkloric medicinal uses of some of these plants to biological activity. Therefore, the use of plant materials to prevent and treat infectious diseases successfully over the years is of high significance for the scientists worldwide [4,5].

The leaf of *E. africanum* is used in the treatment of various ailment which include emetics, as an anti -inflammatory agent, as an analgesic, and also in sore and wound dressing [6]. It is further used to treat chicken pox and gangrenous sores. The leaf decoction of this plant is well-known by the traditional healers in Congo, (Democratic Republic of Congo), Zaire, the Eastern province of Cameroun, and India who used it empirically for several ailment including cardio vascular disease, various inflammation, diabetes, simple

goiter, dysentery, diarrhea, and as an astringent [7]. This plant is reported to contain flavonoids and anthocyanidins and as such was used as a tooth pick for oral hygiene [8,9]. Some alkaloids (pyrolizine alkaloids, PAS) from the leaf of this plant have been implicated to be gastrointestinal tract irritants, cholinesterase inhibitors, and also affect the nervous system by causing drowsiness, salivation, labored breathing, trembling, loss of consciousness, coma, and death due to paralysis [10].

In the northern part of Nigeria (Africa), Gwaska as it is called by the Hausas; the leaf decoction with natron is taken for the treatment of sexually transmitted disease, as an abortificient agent and is also used in the leprosy treatment [11]. Likewise, the aqueous leave extract of this plant is used to cure the blood cancer and mentally related sickness [12]

There is no report available on the phytochemical screening and the assessment of antioxidant potentials of the stem bark of *Erythrophleum africanum*. Therefore, the present study aimed to screen the phytochemical constituents and evaluate the antioxidant potentials of methanolic, acetone, and the aqueous extracts of the stem bark of *Erythrophleum africanum.*

2. Materials and Methods

2.1. Chemicals and reagents

Reagents and other chemicals used in the phytochemical test include: potassium mercuric iodide solution (Mayer's reagent), 0.5 M ammonium hydroxide solution, 5% concentrated sulphuric acid, 0.1% ferric chloride, 5% ferric chloride solution, 5% dilute hydrochloric acid, 1M ammonia solution, analytical grade methanol and acetone (Sigma Aldrich, Germany), 2% glacial acetic acid, 3% acetic anhydride, 5% sodium hydroxide (NaOH), potassium dihydrogen phosphate (KH_2)PO_4, hydrogen peroxide (H_2O_2), 50 mM phosphate buffer pH 7.4, ascorbic acid.Benedict's reagent, Keller–Killani reagent, Salkowski reagent, and Froth reagent (Mon Scientific Nigeria Limited, Lagos).

2.2. Sample collection, Identification, and Preparation

Fresh plant material was collected from Badeggi, Katcha Local Government Areas of Niger State, Nigeria and it was identified with voucher No. 2897, in the Herbarium unit of Biological Sciences Department, Ahmadu Bello University, Zaria. The plant material was air dried and pulverized by using wooded pestle and mortar. The powdered sample was stored in sealed container for further use.

2.3. Sample Extraction

The powdered stem bark sample (450 g) was macerated for (2) two weeks with 1 liter of acetone, methanol, and water (aqueous). To get the crude acetone, methanol, and water extracts, the mixtures were stirred with a magnetic stirrer to dissolve the particles, and then decanted and filtered by using Whatmann No.4 filter paper, and concentrated on rotavapor (R110) at 40 °C. The extracts were kept at room temperature until they were needed.

2.4. Phytochemical analysis

Test for alkaloids

0.5 g of the extract was weighed and transferred into a watch glass before, -1 mL of 5% hydrochloric acid, and then 2 drops of Mayer's reagents were added [13].

Test for flavonoids

5 mL of 1 M ammonia solution were added to a portion of the extract sample followed by addition of 1 mL of 5% H_2SO_4 [13].

Test for tannins (Braymer's Test)

0.5 g of the dried, the powdered sample was boiled in 20 mL of distilled water in a test tube. The resulting mixture was then filtered, and then 2 drops of 0.1% ferric chloride solution was added [13].

Test for phenols

The extracts were dissolved in 5 mL of distilled water, and then 2 drops of 5% ferric chloride solution was added [13].

Test for saponins (Froth Test)

The extracts were diluted with distilled water to 20 mL and were shaken in a graduated cylinder for 15 minutes [13].

Test for quinones

The extracts were diluted with distilled water to 20 mL. 5 mL of the filtrates were hydrolyzed with 1 mL of 5% H_2SO_4. 1 mL of 1 M ammonia solution was added [13].

Test for steroids

2 mL of 3% acetic anhydride was added to 0.5 g of the extract. Likewise, 2 mL of 5% H_2SO_4 was added [13].

2.5. Hydrogen Peroxide Scavenging Assay

The potential of *Erythrophleum africanum* extracts to scavenge hydrogen peroxide was tested by adopting [14]. The concentration of hydrogen peroxide was estimated by absorbance at 230 nm by using a spectrophotometer after preparing a solution of 40 mM hydrogen peroxide in phosphate buffer pH 7.4 (50 mM). The extract (1 mg/mL) in distilled water was added to the hydrogen peroxide solution, and the absorbance was measured 10 minutes later against a blank solution containing phosphate buffer but no hydrogen peroxide; the ascorbic acid was employed at the same concentration for the standard. The following formula was used to compute the percentage of hydrogen peroxide scavenging:

$$\%\ \text{Scavenging}\ (H_2O_2) = \frac{(A0-A1)}{A0}\ X\ 100$$

Where, A_0 is the control absorbance and A_1 is the test absorbance.

3. Results

Table 1. Percentage yields of *Erythrophleum africanum* extracts

Extracts	% yield
Acetone	2.02
Methanol	7.14
Water	5.43

Table 2. Phytochemical screening of *Erythrophleum africanum* extracts

Extraction solvents	Phytochemical(s)							
	Phenols	Flavonoids	Tannin	Saponins	Steroids	Glycosides	Quinones	Alkaloids
Acetone	+	+	+	+	+	+	+	-
Methanol	+	+	+	+	+	+	+	-
Water	+	+	+	+	+	+	+	-

Key: (+) = presence, (-) = absence

Table 3. Antioxidant activities of *Erythrophleum Africanum* extracts

Extracts and standard	Absorbance at 230 nm
Acetone	3.480
Methanol	3.463
Water	3.520
Ascorbic acid	3.500
H_2O_2	3.566

Table 4. H_2O_2 scavenging activity of *Erythrophleum africanum* extracts

Extract	Concentration (mg/mL)	% scavenging
Ascorbic acid	1	1.851
Acetone	1	2.412
Methanol	1	2.888
Water	1	1.289

4. Discussion

4.1. Phytochemical Screening

Cold maceration of *Erythrophleum africanum* powdered stem bark for three days with acetone, methanol, and water yielded 2.02%, 7.14%, and 5.43 %, respectively (Table 1). The standard techniques were used to determine the presence of secondary metabolites such as alkaloids, glycosides, saponins, tannins, quinones, flavonoids, steroids, and phenols in the extracts of *Erythrophleum africanum* (Table 2). These are the primary and secondary metabolites responsible for the medicinal qualities of the plant. The extracts were further subjected to a hydrogen peroxide scavenging test.

Table 1 presents the percentage yield of acetone, methanol, and aqueous extracts of *Erythrophleum africanum* stem bark. The results demonstrate that methanol and water extracts have almost comparable percentage yields, with methanol having a slightly greater percentage yield of 7.14 percent than water and acetone, which have 5.43% and 2.02%, respectively.

4.2. Antioxidant Activity of *Erythrophleum Africanum* Stem Bark Extracts

The result of evaluation of antioxidant activity of *Erythrophleum africanum* stem bark extract shows the antioxidant presence which is higher than the ascorbic acid used as standard except for water extract which is less than the standard, as illustrated in Table 3.

Furthermore, the current work reveals that the antioxidant concentrations were higher in methanol and water compared with the acetone extract; these may be unconnected with differences in polarity, and thus different exorability. Phenolic compounds were linked with antioxidant activity of African and Chinese plants [15]. The best health and nutrition results can be achieved not only from the fruits and vegetables consumption with high antioxidant capacities, but also from medicinal herbs and plants.

Hydrogen peroxide occurs naturally at a low concentration levels in the air, water, human body, plants, microorganisms, food, and beverages. It is widely used as a bleaching agent in textile, paper, and pulp industries. Hydrogen peroxide enters the human body through inhalation of vapor, or mist, and through eye, or skin contact. In the body, hydrogen peroxide is rapidly decomposed into oxygen and water and this may produce hydroxyl radical (OH^-) that can initiate lipid peroxidation and caused DNA damage.

The hydrogen peroxide assay was used as reliable and reproducible parameter to search the *in vitro* general antioxidant activity of pure compound and the plant extract [16]. The decrease in absorbance by the H_2O_2 scavenging assay increase in the concentration of the extract suggested that *Erythrophleum africanum* stem bark extract possess a high antioxidant activity. The methanol, acetone, and water extract of the stem bark was found to be high scavenging for free radicals in which there was no significant difference between the stem bark extract and the standard ascorbic acid at the same concentration, as listed in Table 3. The reducing power of compounds could serve as an indicator

for the potential antioxidant property [17,18,19]. The higher absorbance indicates the strong reducing power potential of the extract as methanol proves in Table 4.

5. Conclusion

Three different stem bark extracts of *Erythrophleum africanum* were subjected to phytochemical screening to identify the bioactive compound(s) present and determine its *in-vitro* antioxidant potentials. Preliminary phytochemical screening of the extracts revealed the presence of seven (7) different secondary metabolites, including phenols, flavonoids, tannins, saponins, steroids, glycosides, and quinones, while alkaloids were absent in both extracts. In the H_2O_2 scavenging assay, the acetone, methanol, and water extracts showed the good dose-dependent free radical scavenging properties. The results showed the presence of antioxidants that were even higher than the ascorbic acid used as a standard, with the exception of the aqueous extract, which is lower than the standard.

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HOW TO CITE THIS ARTICLE

AbdulRazaq Tukur*, Nasiru Malan Musa, Maryam Isma'il Usman, Murtala Yusuf, Tajuddeen Jimoh Abdullahi. Phytochemical Screening and Evaluation of the Antioxidant Potentials of the Stem Bark Extracts of *Erythrophleum Africanum* (African Blackwood). Ad. J. Chem. B, 4 (2022) 202-208.

DOI: 10.22034/ajcb.2022.345261.1123

URL: http://www.ajchem-b.com/article_158150.html