World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

PHYTOCHEMICAL SCREENING, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY *OF* SUDANESE *STEREOSPERMUM KUNTHIANUM (BIGNONIACEAE) AND COMBRETUM GLUTINOSUM (COMBRETUMACEAE)* LEAVES EXTRACTS

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Article Received on 04/12/2023 **Article Revised on 24/12/2023** **Article Accepted on 14/01/2024**

ABSTRACT
The present study is designed to determine the chemical constituents and evaluate the antibacterial and antioxidant activities of *Stereospermum kunthianum* and *Combretumg lutinosum*, aqueous and ethanolic extracts. The results was revealed the presence of tannins, saponins, steroids, triterpenes, flavonoids, Proteins, amino acids and phenolic compounds. Antibacterial test for plants extracts, showed significant activity against two types of bacteria (*Staphylococcus aureus* and *Escherichia coli*) which indicate to possibility of using these plants as antibiotics, while these plants have no effect against fungal. The result of DPPH scavenging activity assay indicates that the plant was potently active.

KEYWORDS: Antimicrobial, antioxidant of *Stereospermum kunthianum* and *Combretumg lutinosum*.

INTRODUCTION
Stereospermum kunthianum (khashkhashabiad) is an African deciduous shrub or small tree found in Congo, Djibouti, Eritrea, Ethiopia, Kenya, Malawi, Senegal, Somalia, Sudan, Tanzania, and Uganda. It is distributed throughout Africa to the Red Sea. *Stereospermum kunthianum* is a small woody tree about 5 to 15 m tall and 25 cm in diameter.[1] In folk medicine pods are chewed with salt used to treat cough, ulcers, leprosy, skin rashes and venereal diseases, while the decoction of the stem bark is used to cure bronchitis, pneumonia, cough, rheumatoid arthritis, and dysentery. The root and leaves have proven useful in the treatment of venereal diseases, respiratory diseases and gastritis. The analgesic and anti-inflammatory effects of the stem bark and the anthelmintic effects of the ethanol extract from the leaves have been reported.[1,2] Scientifically proven therapeutically active substances are present in various medicinal plants from African origin.[1]

Combretum glutinosum (Om habilo) is distributed throughout the Sahelian belt in parts of Senegal, Burkina Faso, Ghana, Mali, Gambia, Niger, Nigeria and Cameroon to parts of Sudan. It grows in a variety of savanna-like forests on many soil types, but does best on sandy and free-draining soils.[3] and it is shrub or small tree that grows up to 12 m tall. It is a deciduous species that sprouts in the middle of the dry season. The bark is grayish-black and may be smooth or rough, with cracks on the upper surface and red to orange slash. The young stems are velvety to tomentose and grayish in color.[3] There are numerous reports of medicinal use of the roots, stems, leaves, bark and fruits. It is used to treat influenza, rheumatism, intestinal worms, cough, colic, impotence, hemorrhoids, constipation, anorexia, malaria, wounds and syphilis.[4]

MATERIALS AND METHODS
Plants material
Two plant materials were obtained from Donki Alhar village (south Alnohud town), West Sudan in January 2018. All plant samples were identified by Dr. Yasmin Adam Ali Aburigal, Department of Horticultural Sciences, Faculty of Agricultural Sciences, University of Gezira. The fresh and healthy leaves were separated instantly and packed in a polyethylene bag. The whole

leaves samples of *Stereospermum kunthianum* and *Combretum glutinosum* were washed with natural water twice and dried under shade at room temperature for 3 days. The samples were transported to the laboratory and kept at room temperature until use.

Extraction

Bioactive materials extracted by maceration techniques, it have been suggested by researchers as more applicable, convenient and less costly method for small and medium enterprises compared to other modern extraction methods.[5] 20 grams of each coarsely powdered plant materials were separately extracted by maceration techniques using 200 ml of ethanol (70%) and distilled water separately in a stopper containers and kept for 72 hours at room temperature. The resulting solutions were filtered and dried at room temperature and weighted then calculated the yield percentage for each extract, the yield was expressed in percentage by dividing the quantity of dry mass obtained after extraction by the dry weight of the powder used before soaking, then kept in containers until use.[6]

Phytochemical Screening

Test for tannins

To 2ml ethanolic and aqueous extracts of each, 2 ml of 10% ferric chloride solution was added in test tubes. Blue-black precipitate was formed and to same amount of gelatin solution was added, white buff color precipitate indicates the presence of tannins.[6]

Test for alkaloids

To 2 ml of each extract 1 ml of 1 % hydrochloric acid was added in each test tube, and heated in a water bath for 10 minutes. 1 ml from the acidified solution was taken and 6 drops of Wagner's reagent / Hager's reagent were added and mixed separately. Brownish-red precipitate / yellow precipitate respectively indicated the presence of alkaloids.[6]

Test for saponins

To 0.5 ml of each extract, 5 ml distilled water was added to each test tubes, and vigorously shaken. Persistent froth produced, checked each 10 minutes for 30 minutes, and indicates the presence of saponins.[6]

Test for cardiac glycosides

To 2 ml of each extract, 1 ml glacial acetic acid, 6 drops of 10% ferric chloride solution and 6 drops of concentrated Sulphuric acid were added to each test tube respectively. Green-blue colour indicates the presence of cardiac glycosides.[6]

Test for steroids

To 2 ml of each extract, 2 ml of acetic anhydride and few drops of concentrated Sulphuric acid were added in test tubes respectively, bluish to green color indicates the presence of steroids.[6]

Test for triterpenes

To 2 ml of each extract, 2 ml of chloroform was added and filtrated. Each one of filtrated treated by few drops of concentrated Sulphuric acid in test tubes. Shaken and allow to stand appearance of golden yellow color indicates the presence of triterpenes.[7]

Test for flavonoid

0.5gms of each plant extract were placed in test tubes and 10 ml of distill water was added, 5 ml of dilute ammonia solution was added, followed by addition of 1 ml concentrated H_2SO_4 to each one tube. Indication of a yellow color shows the presence of flavonoids (Test 1).[8] few quantity of each extract were dissolved in distilled water and filtrated; to each one of these, 2ml of the 10% aqueous sodium hydroxide were added in test tubes respectively, if produce a yellow coloration, then change in color from yellow to colorless on addition of diluted hydrochloric acid which indicated the presence of flavonoids (Test 2).[6]

Test for phenols

Each one of extract was treated with 2ml of distilled water and 10% aqueous ferric chloride solution in test tubes respectively. Blue or green color precipitate indicates presence of phenols.[6]

Test for Proteins and amino acids

Xanthoproteic test: All extracts treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.[7] Ninhydrin Test: To 2ml of each extracts, 0.25% w/v ninhydrin reagent added and boiled for few minutes. Formation of blue color indicates the presence of amino acids.[7]

Test for reducing sugars

Benedict's test: each one of Filtrates treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.[7] Fehling's Test: each one of Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.[7]

Antimicrobial activity of plant ethanolic extracts

The antimicrobial activity was carried out according to method described by department of pharmaceutics - University of Gezira: The sample was prepared by take 1mg from each extract and dissolve in 1ml of distilled water. Mueller Hinton agar media was prepared. 2-3 drops of pathogen culture media were added in melted agar and mixed well. Sterile Petri dishes was divided into 2 sectors, having the name of anti biotic and anti fungal for each samples. The content of the test tubes poured aseptically to the Petri dishes. The media was left to harden at room temperature. A well was made at the center of each sector. Each well was filled with the antibiotic and anti fungal having the same name of the sector. The plates were left at the bench for 2-3hrs then the plates were incubated in the incubator for 18-21hrs

the diameter of clear zone of inhibition were measured for all samples.

Antioxidant activity of plant ethanolic extracts

This method was carried out according to that described method.[9] Sample stock solutions (1000ppm) was diluted to final concentrations of 250, 125, 50, 10 and 5 µg/ml in methanol. 1.0 ml of a 0.3 mm 2, 2 diphenyl-2-picryl hydrazyl (DPPH) in methanol solution was added to a 2.5 ml solution of the different concentrations of the extracts and allowed to react at room temperature for 30 minutes. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA %), using the formula below:

$$AA\% = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

Methanol (1.0 ml) plus plant extract solutions (2.5 ml) was used as a blank. DPPH solution 1.0 ml; 0.3 mm) plus methanol (2.5 ml) was used as control. Stock solution (1 mg/ml) of rutin was diluted to final concentrations of 250, 125, 50, 10 and 5 µg/ml in methanol used as positive control.

RESULTS AND DISCUSSION

Phytochemical analysis performed on the leaves of *Stereospermum kunthianum* and *Combretum glutinosum* using polar solvents (water and ethanol) revealed the presence of tannins, saponins, steroids, triterpenes, flavonoids, Proteins, amino acids and phenolic compounds., and the absence of alkaloids and cardiac glycosides in all crude extracts. Saponins were found in all crude extracts except the aqueous extract of *C. glutinosum*, while triterpenes were found in the aqueous and ethanolic extracts of *C. glutinosum*, while they were absent in the crude extracts of *S. kunthianum*, amino acids were found only in the leaves of *S. kunthianum*. These constituents have different medicinal activities, which explain the numerous uses of these plants in traditional medicine. The result of DPPH scavenging activity assay in this study shows that the plant was potentially active. This indicates that the plant extract contains compounds capable of donating hydrogen to a free radical to remove an odd electron responsible for the radical's reactivity.[10] Crude extracts of *S. kunthianum* and *C. glutinosum* contain tannins and are known to be useful in treating inflamed or ulcerated tissues, and they have remarkable activity in cancer prevention and control,[11,12] Therefore, these plants can serve as a potential source of bioactive compounds in the treatment of cancer. Flavonoids serve as health-promoting compounds due to their anion radicals. These observations support the usefulness of these plants in folk medicine in the treatment of stress-related ailments, bruises, cuts, and wounds,[13] In addition, the plant extracts were found to contain saponins, which are known to have anti-inflammatory activity,[14] and are among the main constituents in traditional Chinese medicine, thus being responsible for most of the observed biological effects [15], which seems to justify the use of *S. kunthianum* and *C. glutinosum* in traditional medicine. The plant extracts were also positive for steroids, which are very important compounds, mainly because of their relationship with compounds such as sex hormones,[16] The presence of these phenolic compounds in these plants contributed to their antioxidant properties and thus the usefulness of these plants in herbal medicine. Phenols have been found to be useful in the production of some antimicrobial compounds such as Dettol and Cresol. This plant is routinely used by many tribes in Africa for the treatment of various diseases.[17]

Antibacterial tests show significant activity against two types of bacteria (*Staphylococcus aureus* and *Escherichia coli*), indicating the possibility of using these plants as antibiotics, while these plants have no activity against Fungal. Use of water as a solvent to simulate the traditional method used by humans and use of ethyl alcohol to confirm the presence of polar phytochemicals, moreover, ethyl alcohol is available, the best solvent and has a low toxicity.

Table (1): Yield percentage of crude plants extracts.

Crude extract	Yield (%)	Crude extract	Yield (%)
S. *kunthianum* water extract	15.0	*C. lutinosum* water extract	23.0
S. *kunthianum* ethanolic extract	22.5	*C. lutinosum* ethanolic extract	28.5

% = wt. of crude extract / wt. of sample x 100

Table (2): Phytochemical screening of aqueous and ethanolic crude extracts for two plants.

Secondary metabolite	Name of test	S. *kunthianum* H_2O	*C. lutinosum* H_2O	S. *kunthianum* EtOH	*C. lutinosum* EtOH
Tannins	Ferric chloride	+	+	+	+
	Gelatin test	-	+	-	+
Alkaloids	Wagner's test	-	-	-	-
	Hager's test	-	-	-	-
Saponins	Foam test	+	-	+	+
Cardiac glycoside	Keller-Kilani test	-	-	-	-
Steroids	Liebermann-Burchard reaction	+	+	+	+
Triterpenes	Salkowski's test	-	+	-	+

Flavonoids	Test 1	+	+	+	+
	Test 2	+	+	+	+
Phenols	Ferric chloride	+	+	+	+
Proteins and amino acids	Xanthoproteic test	+	+	+	+
	Ninhydrin test	+	-	+	-
Reducing sugars	Benedict's test	+	+	+	+
	Fehling's test	+	+	+	+

Plus (+) indicates the presence minus (-) signifies absence.

Table (3): Antimicrobial activity (inhibition level %) of the plants ethanolic extracts.

Micro-organisms	EtOH Extract		Control	
	S. kunthianum	***C. glutinosum***	**Gentamicin**	**Nystatin**
Staphylococcus aureus	27	16	35	-
Escherichia coli	16	14	24	-
Candida albicans	0.0	0.0	-	25

Table (4): DPPH scavenging activity of plants ethanolic extracts.

Plants extracts	Concentration	Scavenging activity
S. *kunthianum*	250µg/ml	95.6%
	125µg/ml	93.7%
	50µg/ml	93.3%
	10µg/ml	86.7%
	5 µg/ml	63.3%
C. lutinosum	250µg/ml	95.3%
	125µg/ml	95.3%
	50µg/ml	93.3%
	10µg/ml	64.4%
	5 µg/ml	53.3%

Table (5): DPPH scavenging activity of plants ethanolic extracts.

Plants extracts	EC_{50}
S. *kunthianum*	47.0
C. lutinosum	47.4
Rutin (standard)	12.0

Disclosure statement

- **Conflict of interests:** The authors declare that they have no conflict of interest.
- **Author contributions:** All authors contributed equally to this work
- **Ethical approval:** All ethical guidelines have been adhered.
- **Sample availability:** Samples of the plants material are available.

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