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Phytochemical Investigation, Antimicrobial, Antioxidant and Anti-Diabetic Potential of *Guiera Senegalensis* Leaves Extracts

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ABSTRACT

The aim of the current study was to detect phyto constituents present in GuieraSengalensis extracts using biochemical tests and to evaluate antimicrobial, antioxidant and anti-diabetic potential of the four leaves extracts of the plant using cup-plate diffusion method, DPPH free radical scavenging assay and glucose uptake in yeast cell assay. The plant showed the presence of alkaloid, flavonoid, tannins, sterol, saponin and glycosides. The ethanol extract showed high antimicrobial activity against E.coli with inhibition 30 mm at concentration 100 mg/ml. The plant showed good antioxidant activity with minimum radical scavenging activity 12.3% for petroleum ether extract and maximum activity 74.1% for methanol extract in comparison with standard propyl-gallate 77%. The methanolic extract showed good anti-diabetic activity with minimum increase 25.85 at 40ug/ml and maximum 58.3 at 80ug/ml glucose concentration. The result obtained revealed that this plant is very important from medicinal point of view.

INTRODUCTION

Medicinal plants are an important part of our natural wealth. They are therapeutic agent as well as valuable raw materials for manufacturing numerous traditional and modern medicine.

Plants that possess therapeutic properties or exert beneficial pharmacological effects on the human body are generally designated as medicinal plants. The medicinal plants have been used for treatment of illnesses and diseases, since the dawn of time. Researchers have found that people in different parts of the world tend to the same or similar plants for treating the same illnesses. Recently, World Health Organization (WHO) estimated 80% of people worldwide rely on herbal medicines partially for their primary health care.

Guiera Senegalensis locally named as Gabeish, are Semi-shrub up to 3-5 m tall with spindly bole or many branched from the base, all parts covered with black glandular dots, bark scaly, more or less smooth to finely scaly, grey to brown.

Guiera Senegalensis widely distributes in the Savannah region of West and Central Africa in Senegal, Gambia, Mali, Niger, Burkina Faso, Guinea-Bissau, Guinea, Chad, Mauritania and Sudan.

Guiera Senegalensis is one of the most popular West Africa medicinal plants, and is used to treat awide variety of diseases. The plant leaves sometimes combined with other species, is drunk to treat dysentery, diarrhea, colic, gastroenteritis, beriberi, rheumatism, hypertension, eczema, epilepsy, leprosy, impotence, venereal diseases, malaria fever, cough, colds asthma, bronchitis and tuberculosis.

Guiera Senegalensis is belong to family *Combretaceae*, the previous phytochemical screening showed the presence of sterol, triterpenes, glycosides, flavonoid, alkaloid and tannins (El Ghazali, 1997).

MATERIALS AND METHODS

Plant Collection and Identification

Guiera Senegalensis were collected by hand in October 2018 from West Kordofan State (Alnehwood) Susan. Then the plant was identified by herbarium of Medicinal and Aromatic Plant Institute, National Centre for Research.

Plant Preparations

Guiera Senegalensis leaves were dried in shadow; for 3 days then were grinded by using mortar, then plant was weight with electric balance.

Extraction

The powered plant material (1 kg) were weight and extracted successively with petroleum ether, chloroform, methanol and ethanol and yield percentage was calculated.

Phytochemical Screening

Phytochemical screening of four extracts of *Guiera Senegalensis* leaves was performed using standard procedures as described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973).

Alkaloid

- Wagner's test: To 2 ml of extract, 1 ml of Wagner's reagent was added, the appearance of reddish-brown precipitate indicates presence of alkaloid.
- Hager's: To 2 ml of extract 1 ml Hager's reagent was added, the appearance of yellow precipitate indicates the presence of alkaloid.
- Dragendroffs's: To 2ml of extract, 1 ml of Dragendroffs reagent was added the appearance of brick red precipitate indicates presence of alkaloid.

Flavonoid

- Ammonium solution test: To 2 ml of filtrates 1 ml of dilute ammonia solution 1% was added. The appearance of yellow color indicates presence of flavonoid.
- Shinoda test: To 2 ml of extract was dissolved in the ethanol then divided in to 2 test tube.
- In the first test tube 1 ml of sodium hydroxide 10% was added. The appearance of yellow color indicates the presence of flavonoid.
- In the second test tube: 0.3 g of magnesium powder, 5 drops of HCl was added then heated in water bath for 10 minutes the red or pink color was formed indicates the presence of flavonoid.

Tannins

- Ferric chloride test: To 2 ml of extract dissolved in ethanol then 0.5 ml of ferric chloride 5% was added. The blue-black color indicates the presence of tannins.
- Legal test: 2ml of extract 0.3 ml of lead acetate solution was added creamy gelatinous precipitates indicate the presence of tannins.

Glycosides

- killer-killiani test: 2 ml of extract 1 ml of glacial acetic acid 3 drops 5% Ferric chloride and concentrated suphuric acid were added. The reddish-brown color at the junction of two layers and bluish green in upper layer indicates the presence of glycosides.

Saponin

- Foam test:The extracts was diluted with 2ml of distilled water and shaken vigorously and observed for persistent foam, which indicates the presence of saponin.

Terpenoids and Sterols

- Salkowaski test: To 2 ml of extract, 2 ml chloroform and concentrated sulphuric acid was added, shaken and allow to stand. Appearance of greenish blue color indicates the presence of terpenoids and sterols.
- Liebermannbur chard test: To 2 ml of methanolic plant extract mix with chloroform, 1-2 ml of acetic anhydride was added. Then 2 drops of concentrated sulphuric acid was added from the sides of test tube, appearance of greenish blue color indicates the presence of terpenoids and sterols.

Antimicrobial Activity Test

Cup plate diffusion method:or hole diffusion method as modified by Ali et.al. (2014) was the standard method used to determine the antibacterial and antifungal activity of the bioactive compounds. Solving Briefly in this method: sterile nutrient agar powder was prepared by dissolving 12 agars in 250 ml distilled water, boiled to ensure complete dissolution and sterilized at 121℃ for 30 minutes and dispensed in to labeled petri-dishes and allowed to gel. The sterile agar plates were inoculated with the test culture by surface spreading uses wing 6 mm sterile cork borer. 0.2 g of each crude extracts was weighed in to sample bottles and dissolved in to 100 ul of DMSO and then the concentration 100 mg/ml, 50 mg/ml, 25mg/ml,12.5mg/l were sterilized dilution by DMSO and 5 ul of DMSO was used for positive control. The agar was inoculated with the test organism using a sterile swab stick before incubating at 37℃ for 24 hours. Zones of inhibition were determined by measuring the diameter of inhibition zone around the well in mm including the well diameter.

Free Radical Scavenging Procedure (DPPH)

This method was carried out according to that described by (Shyur et.al.,2005). with some modifications stock solution was prepared by dissolving 1 mg of the sample in 1 ml of absolute ethanol (98%). Stock solution was diluted to final concentration 100, 50, 25, 12.5, 6.25, 3.125.1.526 ug/ml in ethanol. 0.9 ml tris-HCl and 1 ml of 0.1 mm DPPH in methanol solution were added to each concentration and incubated at room temperature in dark for 30 minutes. The absorbance of the resulting mixture was measured at 517 nm and converted to percentage antioxidant activity using formula below:

Scavenging activity (%)

$$= \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Anti-Diabetic Activity

The anti-diabetic activity of extract was measured on the basis of glucose uptake in yeast cell method. Commercial baker's yeast was dissolved in distilled was waster it was kept overnight at room temperature the yeast cell suspension was washed by centrifugation at 2400 rpm in distilled water for 5 minutes. The process wall was repeated again and again until clear supernatant fluid was obtained. 1% suspension of yeast cells with distilled water was prepared 5 ml glucose solution was prepared in distilled water 1 mg of the extract was dissolved in DMSO before stock solution various concentration (10,20,40,60,80 ug) in DMSO. 1ml glucose and 100 ul of yeast was used to prepare reaction mixture was cortexes and incubated further for 60 minutes at 37℃, after one hour of incubation of the reaction mixture the tubes were centrifuge for 5 minutes at 3800 rpm. Glucose left behind in the supernatant was estimated by measuring the absorbance via spectrophotometer at 520 nm. The percentage increase in uptake was calculated by formula:

%increase in glucose =

$$\frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Results and Discussion

Table1. *Phytochemical Screening of Guiera Senegalensis*

Secondary metabolites	Test	Solvent of extraction			
		P. E	ChCl3	MeoH	EtoH
Alkaloid	Dragendroffs	+	+	+	+
	Wagner's	+++	+	+++	+++
	Hager's	++	+	+++	+++
Flavonoids	Ammonia	+++	+	-	+++
	NaOH 10%	++	+++	-	+++
	Mg/H_2So_4	-	-	-	-
Tannins	$FeCl_3$		+	+++	+++
	leadacteate	+++		+++	+++
Sterols	Salkowaski			+++	+++
	Liebermann	+++		+++	++
Triterpenes	Salkowaski	-	-	-	-
	Liebermann	-	-	-	-
Saponin	Foam test		+	++	+++
Glycoside	Killer-killiani	++	+++	+++	+++

P.E: petroleum ether ChCl3: chloroform MeOH: Methanol EtoH: ethanol+++ means high, ++ meansmoderate, + means low, - means not detect

Table2. *Guiera Senegalensis Antimicrobial Activity*

Solvent of extraction	concentration	Staphyocous aureus	E. Coli	Candida albicans
	100 mg/ml	21 mm	10mm	20mm
	50	17	7	18
	25	15	6	16
	12.5	13	4.5	14
	100	12	24	8
	50	8	17	6
	25	6	13	4

	12.5	5	10	2
	100	19	28	19
	50	12	19	10
	25	8	17	8
	12.5	6	16	6
	100	17	30	16
	50	13	26	11
	25	8	22	8
	12.5	6	14	6

Table3.*Guiera Senegalensis Antioxidant Activity*

SAMPLE	%RSA
Ethanol extract	69%
Methanol extract	74.1%
Chloroform extract	48%
Petroleum ether	12.3%

%RSA: percentage radical scavengingNote: propyl-gallate as standard %RSA= 77%

Table4. *Guiera Senegalensis Anti-Diabetic Activity*

Extract	40 ug/ml	60 ug/ml	80ug/ml
Methanol	25.85	56.46	58.3
Ethanol	-	17.15	38.25
Chloroform	-	26.6	36.6
Petroleum ether	-	33.77	39.48

Note: Metronidazole as standard:48% at 10 ug/ml and 80 % at 80 ug/mlPhytochemical Screening of Guiera Senegalensis listed in table (1).

Antimicrobial Activity

In present study, four extracts of *Guiera Senegalensis* leaves extracts were screened for antimicrobial activity on one Gramm positive bacterium *Staphylococcus aureus* and One Gramm negative bacterium *Escherichia coli* and one fungus *Candida albicans*. The result showed in table (2). The degree of antimicrobial activity was classified as low, medium or high depending on the diameters of inhibition zones. These zones diameters develop as a result of amount of the herbal extract, the concentration of the active ingredient in the inoculums and the resistance of the microbes to antimicrobial constituent in the extract.

Antioxidant Activity

The four extracts of *Guiera Senegalensis* were subjected to DPPH free radical scavenging assay to evaluate their antioxidant activities with reference the propyl-gallate. (Table3). The result obtained showed that*Guiera* methanol extract has a good activity with %RSA 74.1% in comparison with reference %RSA 77%.

Anti-Diabetic Activity

The evaluation of antidiabetic potential of medicinal plant can be exposed in vitro by a number of procedures such as study of glucose uptake, inhibition of alpha amylase, alpha glycosidase enzymes and effect on glycoslation of hemoglobin.

For assessing the hypoglycemic properties of different medicinal plants, the method of glucose transport through the yeast cell membrane has been achieved an outstanding importance as an in vitro screening method. The result revealed that methanolic extract of *Guiera Senegalensis* has good antidiabetic activity.

Conclusion

Guiera Senegalensis leaves extracts were subjected to antimicrobial assay using cup plate diffusion method. The plant also subjected to antioxidant scavenging assay using yeast cell method. All these activities belong to class of phytoconstituents present in the plant. Which confirmed the uses of plant in folk medicine.

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