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Phytochemical Analysis and Antioxidant Evaluation of Methanolic Extract of *Senna occidentalis* and *Guiera senegalensis* leaves

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Abstract In the present study, the research is concerned with the extraction using soxhlet extraction technique in solvent with increasing polarity. The phytochemical constituents of *Senna occidentalis* and *Guiera senegalensis* were identified using various test methods and evaluates the antioxidant activity of methanolic extract of the plants leaves. The antioxidant activity of methanolic extract of both *Senna occidentalis* and *Guiera senegalensis* leaves were determined by DPPH (2,2-diphenyl-1-picrylhydrazine) free radical scavenging capacity with varying concetration. The antioxidant activity of the methanolic extract for both plants showed significantly potential in the DPPH radical reaction system. The radical scavenging potential, expressed as percentage inhibition of *Senna occidentalis* (leaves) and *Guiera senegalensis* (leaves) with respect to DPPH radical was found to be between 43.468 – 72.564% and 47.748 – 66.154% respectively. The percentage scavenging activities of the samples were express in 50% minimum inhibitory concentration (IC_{50}). The 50% inhibitory concentration (IC_{50}) of *Guiera senegalensis* was found to be 58.550 µg/ml which was significantly lower than the IC_{50} in *Senna occidentalis* (77.565 µg/ml).

Keywords *Senna occidentalis, Guiera senegalensis,* antioxidants, phytochemical, DPPH

1. Introduction

A medicinal plant is a plant that is used to maintain health, and to be administered for a specific condition, or both, whether in modern medicine or in traditional medicine [1, 2]. Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesized hundreds of chemical compounds for functions including defence against insects, fungi, diseases, and herbivorous mammals. Numerous phytochemicals with established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Further, the phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain assessed by rigorous scientific research to define efficacy and safety [1]. The compounds found in plants are of many kinds, but most are in four major biochemical classes: alkaloids, glycosides, polyphenols, and terpenes.

Plants are to generate or produce a numerous number of diverse bioactive compounds [3]. Free radical damage may be protected by high concentration of phytochemicals accumulated in several part of the plant [3]. Beneficial phytochemicals contained in plants may enhance the need for the human body by acting as antioxidant [4]. Studies shows that some plants are source of antioxidants, like phenolic compounds such as tannins, flavonoids and lignins

found in plants which all act as antioxidant. Many antioxidants play an important role in reducing inflammation, delaying aging and preventing cancers [3]. Virtually antioxidant reduces the oxidative damage in food by inhibiting oxidation caused by reactive oxygen species (ROS), specifically increasing the quality of these food [5]. Reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), hydroxyl radical (OH•), superoxide anion (O_2^-) and nitric oxide (NO) are deleterious to various physiologically important molecules including proteins, lipids and DNA [6]. ROS, generated in living organisms during metabolism, are very unstable and highly reactive, and they tend to initiate chain reactions which result in irreversible chemical changes in proteins or lipids. These deleterious reactions can result in cellular dysfunction and cytotoxicity. A number of cellular defence systems have evolved to counteract the accumulation of ROS. They include reducing agents such as β-carotene, vitamin C, E and ascorbic acid, as well as enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione and peroxidases [7], and therefore exert their protective role by being oxidized themselves. Furthermore, many antioxidants compounds have been characterized from plants including flavonoids. Flavonoids are phenolic compounds with important roles in scavenging free radicals and thus play vital roles in preventing oxidative stress associated disorders [8].

Hence, the present study report, the phytochemical screening and evaluation of antioxidant activity of *Senna ocidentalis* and *Guiera senegalensis* leaves.

Material and Methods

All solvent used were of high analytical grade and purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA).

Sample Collection

Both plant leaves were collected from Federal University Dutse (F.U.D) Jigawa State, Nigeria. And were authenticated at the herbarium unit at the Biological Sciences Department, Ahmadu Bello University Zaria-Nigeria, and they were deposited and voucher numbered19195 for *Senna occidentalis* and 19196 for *Guiera senegalensis* were assigned to the specimens.

Both plant leaves were chopped into pieces using iron knife, oven dried at 80^{o}C for one (1) hour, pulverised using mortar and pestle, and sieved using a 600 MICS size siever and then stored in a non-absorptive nylon for subsequent use.

Extraction of Plant Material

The plant materials were extracted using a soxhlet extractor successively in n-hexane, diethyl ether, chloroform, ethyl acetate and methanol exhaustively for 12 hours; 1 hour; 8 hours; 10 hours; and 12 hours respectively for both plant materials until complete extraction. The solvents were removed and concentrated using a rotary evaporator and stored in a screw cap bottles at 0^{o}C and labelled until usage.

Preliminary Phytochemical Screening

The extracts were subjected to various phytochemical tests to identify the constituent secondary metabolites using standard methods [9, 10] with some modifications. The metabolites that were tested for includes: Anthraquinones, Alkaloids, Carbohydrates, Cardiac glycosides, Flavonoids, Saponnins, Steroids, Tannins, Terpenes.

Test for Anthraquinones

To 10mL of benzene small quantity of the extract was added and shaken, the content was filtered and 5ml of 10% ammonia solution was added to the filterate then, the mixture was shaken. No change in colour were observed in the ammoniacal layer (lower phase) which indicated the presence of free anthraquinones [11].

Test for Alkaloids

To each sample (0.5g) in test tube was added 5ml of 1% hydrochloric acid and stirred on a water bath and filtered. The filterate was divided into three aliquots. To the first test, 3 drops of freshly prepared Dragendoff's reagent was

added. An orange to brownish precipitate indicated the presence of alkaloid. To the second test tube 1 drop of Mayer's reagent was added. A yellowish colour precipitate indicated the presence of alkaloid. To the third test tube 1 drop of Wagner's reagent was added. A reddish- brown precipitate indicated the presence of alkaloid [12].

Test for Cardiac glycoside (Kella-Killani test)

5ml of glacial acetic acid containing trace of ferric chloride was added to 0.5g of the extract in 20ml test tube. The test tube was held at 45^o and 1ml concentrated H_2SO_4 was carefully added down the side. A purple ring colour at the interface indicated the presence of cardiac glycoside.

Test for Flavonoid (Shinoda test)

5ml was added to 1g of sample in 20ml test tube, to the solution three pieces of magnesium chips was added followed by few drops of concentrated HCl. A purple colour indicated the presence of flavonoid [10].

Test for saponnins (Frothing Test)

About 0.1g of the extract was shaken with water in a test tube. Frothing was observed which persisted for 1 minute that indicated the presence of Saponnins [12].

Test for steroids/Terpenes (Liebermann-Buchard Test)

1ml of chloroform was added to each 0.5g of sample in 20ml test tube and to the solution few drops acetic anhydride were added followed by addition of concentrated $H_2SO$and carefully mixed thoroughly. A blue colour that change with time indicated the presence of steroids/terpenes [12].

Test for Phenolic compounds and Tannins (Ferric Chloride Test)

To 1 g of each sample in 20 ml test tube was added 5 ml of distilled water and boiled and the mixture was filtered. Two drops of ferric chloride were added to the filtrate, formation of green precipitate was observed which indicated the presence of tannins [13].

In vitro DPPH Free Radical Scavenging Activity

The antioxidant activity of methanolic extract of both *Senna occidentalis* and *Guiera senegalensis* leaves were determined by DPPH (2,2-diphenyl-1-picrylhydrazine) free radical scavenging capacity. Extracts were dissolved as 500µg/ml in methanol. Different concentration of the solution such as 40, 80, 120, 160 and 200µg/ml was obtained using serial dilution and each concentration was mixed with 0.5ml of a DPPH – methanol solution (1mM). These samples were shaken well and kept in an incubator at room temperature for 30min activity [14]. The absorbance was measured at 517nm. The scavenging activity on the DPPH radical was calculated using the following equation:

$$\% \text{ inhibition} = \frac{A_0 - A_i}{A_0} \; X \; 100$$

Where A_0 is the absorbance of the control and A_i is the absorbance of the sample. Ascorbic acid was used as a standard control. Scavenging activity was expressed as IC_{50}, which represent the concentration of the extract (µg/ml) required to inhibit 50% of the free radical scavenging activity [14].

Results and Discussion

Weight and percentage yield of leaves extracts

For the recovery of secondary metabolites from the plants sources in which extraction is the main step. The extract yield depends on some factors like nature of the solvent with varying polarity, extraction method, nature of phytochemicals, presence of interfering substances and solvent to sample volume ratio e. t. c. the commonly used solvents for polar compounds extraction include alcohol (methanol and ethanol), ethyl acetate, acetone and mixture of methanol or ethanol and water. In present study the solvents used are methanol, ethyl acetate, chloroform, diethyl

ether and hexane. Among all the solvent used a greater yield in both plants leaves were obtained in hexane and methanol.

Table 1: Result of extraction

S. No.	Sample	Weight used (g)	Solvents Used	Weight of Crude Extract (g)
1	*Senna Occidentalis*/Leaves	400	Hexane	33.20
			Diethyl Ether	3.00
			Chloroform	12.40
			Ethyl Acetate	3.80
			Methanol	24.80
2	*Guiera Senegalensis*/Leaves		Hexane	13.20
			Diethyl Ether	1.80
			Chloroform	8.40
			Ethyl Acetate	7.40
			Methanol	10.60

Qualitative Phytochemical Screening

Reports showed that, the most active phytochemicals in plants include alkaloids, flavonoids, phenols, tannins, saponnins, steroids, glycosides and terpenoids in different concentration which gives their pharmacological actions. Comparative qualitative phytochemical screening of all leave extract were shown in table 2 and 3. All the leaves extract showed the presence anthraqiunones, flavonoids, carbohydrate, cardiac glycosides, saponnins, steroids/tepenes, phenolic compounds and tannins. Although glycosides are present in *Senna occidentalis* but absence in various extract of *Guiera senegalensis* and alkaloids are present in *Guiera senegalensis* but absence in various extract of *Senna occidentalis*.

Table 2: Preliminary phytochemical analysis of leaves extract obtained from *Senna occidentalis*

Metabolites	Test Used	Leaves Extract				
		N-HX	DE	CF	EA	ME
Anthraquinones	General test					
Free anthraquinones		-	-	-	+	+
Combined anthraquinones		-	-	-	+	+
Alkaloids	Dragendoff's test	-	-	-	-	-
	Mayer's test	-	-	-	-	-
	Wagner's test	-	-	-	-	-
Carbohydrates	Molisch's test	+	-	-	-	+
Cardiac glycosides	Kella-Killani test	+	+	+	-	-
Glycosides	$FeCl_3$ test	-	-	-	-	+
Flavonoids	Shinoda test	-	-	-	-	+
	NaOH test	-	-	-	-	+
	Sulphuric acid test	-	-	-	-	+
	Lead acetate test	-	-	-	-	+
Saponnins	Frothing test	-	-	-	+	+
Steroids/ Terpenes	Liebermann-Buchard test	+	+	+	+	+
	Salkowski test	+	+	+	+	+
Phenolic compounds and tannins	$FeCl_3$ test	-	-	-	-	+

n-HX= n-Hexane, DE= Diethyl Ether, CF= Chloroform, EA= Ethyl Acetate, ME= Methanol

Table 3: Preliminary phytochemical analysis of leaves extract obtained from *G. senegalensis*

Metabolites	**Test Used**	**Leaves Extract**				
		N-HX	**DE**	**CF**	**EA**	**ME**
Anthraquinones (free anthraquinones)	General test	-	-	-	+	+
Alkaloids	Dragendoff's test	+	+	+	+	+
	Mayer's test	+	+	+	+	+
	Wagner's test	+	+	+	+	+
Carbohydrates	Molisch's test	+	-	-	-	+
Cardiac glycosides	Kella-Killani test	+	+	+	+	+
Glycosides	$FeCl_3$ test	-	-	-	-	-
Flavonoids	Shinoda test	+	+	+	+	+
	NaOH test	+	+	+	+	+
	Sulphuric acid test	+	+	+	+	+
	Lead acetate test	+	+	+	+	+
Saponnins	Frothing test	-	-	-	+	+
Steroids/ Terpenes	Liebermann-Buchard test	-	-	-	+	+
	Salkowski test	-	-	-	+	+
Phenolic compounds and tannins	$FeCl_3$ test	-	+	+	+	+

N-HX= N-Hexane, DE= Diethyl Ether, CF= Chloroform, EA= Ethyl Acetate, ME= Methanol

Table 4: Sample A and Control Absorbance, Mean, Standard deviation and Percentage Scavenging

Concentration (µg/ml)	**Absorbance of sample (nm)**			**Absorbance of control (nm)**	**Mean (nm)**	**Standard deviation**	**Percentage scavenging (%)**
40	0.251	0.252	0.249	0.444	0.251	±0.002	43.468
80	0.212	0.214	0.210	0.427	0.212	±0.002	50.351
120	0.177	0.176	0.178	0.418	0.177	±0.001	57.656
160	0.142	0.143	0.144	0.406	0.143	±0.001	64.778
200	0.105	0.107	0.108	0.390	0.107	±0.001	72.564

Sample A = *Senna occidentalis*
Control = Ascorbic Acid

Table 5: Sample B and Control Absorbance, Mean, Standard deviation and Percentage Scavenging

Concentration (µg/ml)	**Absorbance of sample (nm)**			**Absorbance of control (nm)**	**Mean (nm)**	**Standard deviation**	**Percentage scavenging (%)**
40	0.232	0.230	0.234	0.444	0.232	±0.002	47.748
80	0.197	0.200	0.203	0.427	0.200	±0.003	53.160
120	0.170	0.172	0.171	0.418	0.171	±0.001	59.091
160	0.148	0.146	0.149	0.406	0.148	±0.002	63.547
200	0.130	0.132	0.133	0.390	0.132	±0.002	66.154

Sample B = *Guiera senegalensis*
Control = Ascorbic Acid

Result of DPPH assay of Methanolic leaf extracts of *Senna occidentalis* and *Guiera senegalensis*

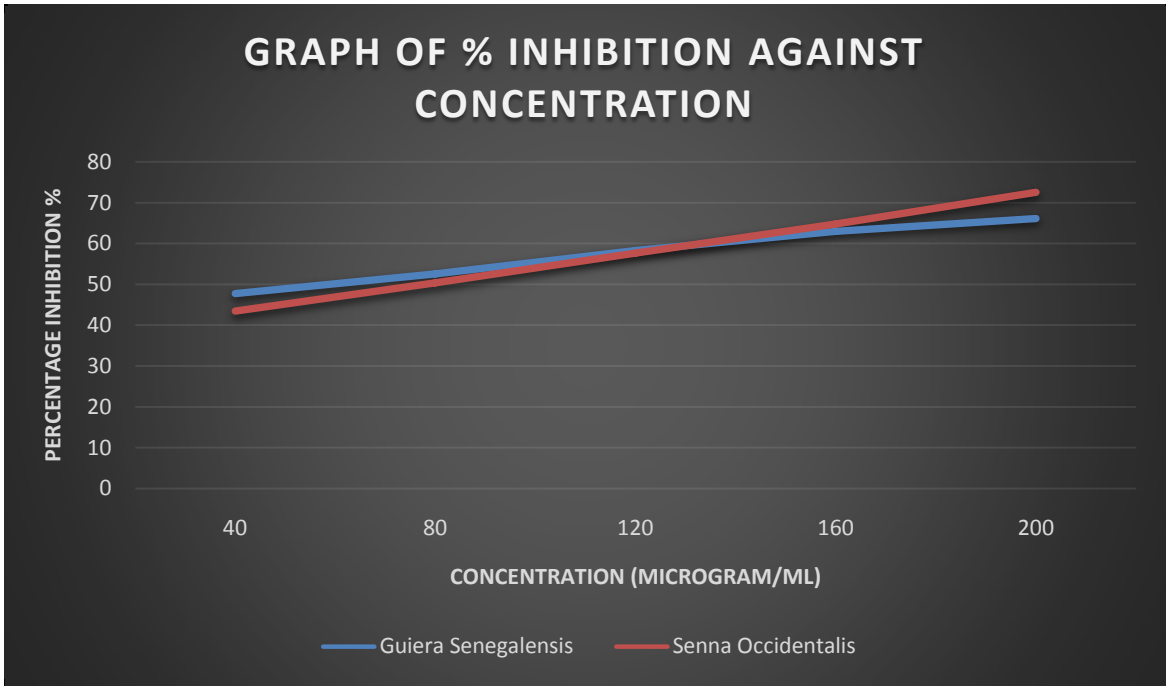

Figure 1: DPPH Radical Scavenging Activity (%) of both Guiera senegalensis and Senna occidentalis versus Concentration (µg/ml)

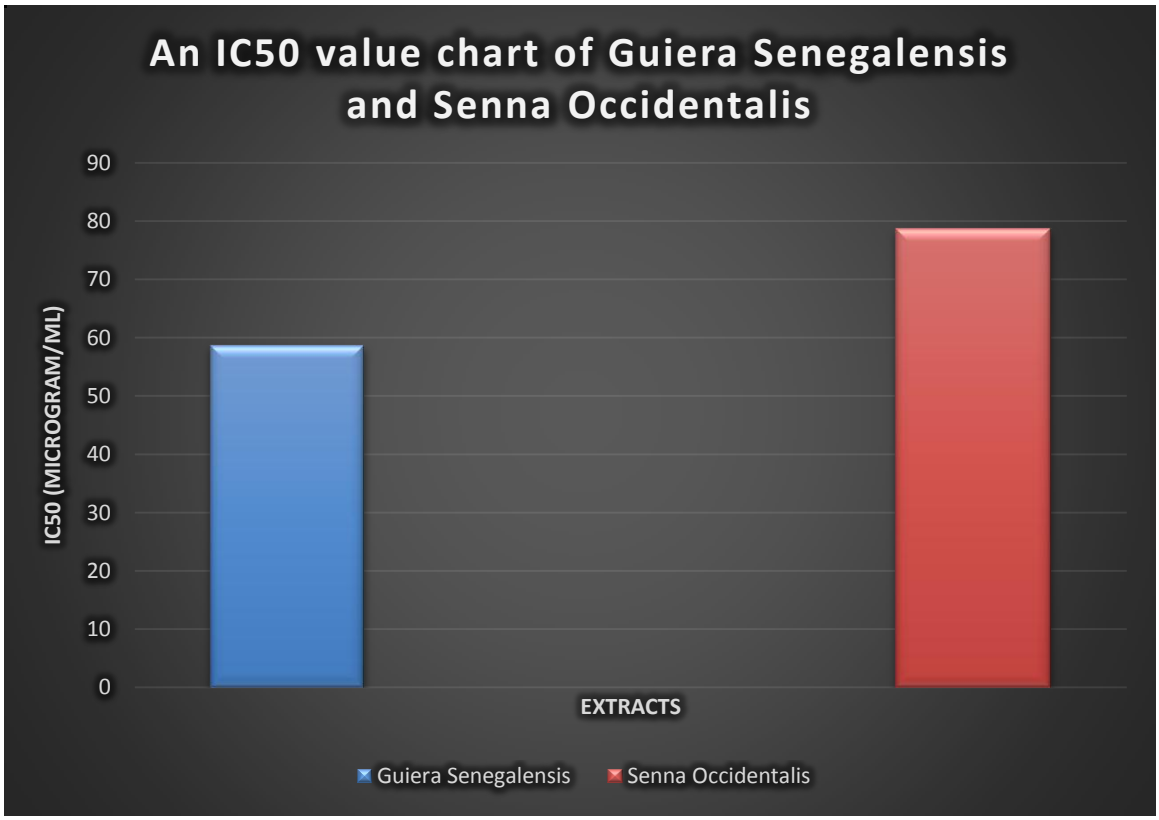

Figure 2: Graphical Presentation of the IC_{50} value of Guiera senegalensis and Senna occidentalis leaves Extracts

Discussion

From the result of extraction shown in table 1 above, among all the solvents hexane was found to have highest mass for both *Senna occidentalis* and *Guiera senegalensis* leaves extract using the aforementioned successive soxhlet extraction technique.

The preliminary phytochemical screening reveals the presence of anthraquinones (free anthraquinones and combined anthraquinones), carbohydrates, cardiac glycosides, flavonoids, saponnins, steroids/terpenes, phenolic compounds and tannins for both *Senna occidentalis* and *Guiera senegalensis* leaves extract and absence of alkaloids for various extract of *Senna occidentalis* using different test as shown in table 2 above. While on the other hand glycosides was found to be absence for various extract of *Guiera senegalensis* using different test as shown in table 3 above.

The antioxidant activity of the methanolic extract for both plants showed significantly potential in the DPPH radical reaction system. The radical scavenging potential, expressed as percentage inhibition of *Senna occidentalis* (leaves) and *Guiera senegalensis* (leaves) with respect to DPPH radical was between 43.468– 72.564% and 47.748 – 66.154% respectively (Table 4 and 5). The percentage scavenging activities of the samples were express in 50% minimum inhibitory concentration (IC_{50}). The 50% inhibitory concentration (IC_{50}) of *Guiera senegalensis* was found to be 58.550 µg/ml which was significantly lower than the IC_{50} in *Senna occidentalis* (77.565 µg/ml).

The above data illustrates that, the percentage of free radical inhibition increases as the concentration increased in both extracts and also, absorbance of the DPPH decreases as the concentration increases because, the stable radicals generated in the solution are scavenged by the antioxidants, thus changing the dark violet-like colour of the reaction mixture to a pale-yellow colour.

Conclusion

Medicinal plants are the source of secondary metabolites. From the result we obtained it can be concluded that, the leaves of both plants were found to be rich in secondary metabolites (phytochemicals). Those result also clearly shows that, the methanolic extract of both *Senna occidentalis* and *Guiera senegalensis* leaves were effective against free radical mediated diseases, this indicates that, the leaves of both plants can be used as free radical scavenging agent and antioxidant as well as aids in the treatment of diseases mediated by reactive oxygen species (ROS) among others, especially the leaves extract of *Senna occidentalis* which shows relatively higher IC_{50} value and % scavenging than that of *Guiera senegalensis.*

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