Open Access Journal of Chemistry
Volume 3, Issue 3, 2019, PP 7-12
ISSN 2637-5834

Phytochemical Analysis and Thin Layer Chromatography Profiling of Crude Extracts from GuieraSenegalensis (Leaves)

Lawal A.M.[1*], Lawan M. M.[2], Apampa S. A[3]

[1]Department of Chemistry, Federal University Dutse, Jigawa State, Nigeria.
[2]Department of Biological Sciences, Federal University Dutse, Jigawa State, Nigeria.
[3]Department of Chemistry, Federal College of Education, PMB 1041, Zaria.

***Corresponding Author:** Lawal A.M, Department of Chemistry, Federal University Dutse, Jigawa State, Nigeria. E-mail: abbulbakr@gmail.com*

ABSTRACT

Medicinal plants, which are also referred to as medicinal herbs, since plants produces bioactive chemical compounds (phytochemicals) and these are used for medicinal practices since prehistoric stone ages, this research however, is concerned with the extraction using Soxhlet extraction technique, phytochemical screening using various test methods, which reveals the presence anthraquinones (free anthraquinones and combined anthraquinones), alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponnins, steroids/ terpenes, phenolic compounds and tannins were present and absence of glycosides were recorded, and also, thin layer chromatography profiling which gives probable foundation for further structural elucidation amongst others. This research shows the presence of potent secondary metabolites present in the leaves of guierasenegalensis(leaves).

Keywords: *Medicinal herbs, Medicinal plants, Phytochemicals, Drug Development, Chromatography*

INTRODUCTION

A medicinal plant is any plant material be it seeds, root extracts or leaves extract that is used to cure or fight against infection or attempt to maintain health, which are to be administered for specific ailments which can either be in modern or traditional medicine, this definition of medicinal plant is also supported by [1,2]
Guierasenegalensis is a flowering plant species in the family combretaceae and genus *guiera*, a dwarf perennial shrub distributed around various parts of Africa and Asia [3]. Family Combretaceae are trees or shrubs, comprising of about 20 genera and 500 species. These species distributed around the globe and central of diversity been in Africa and Asia. *G. senegalensis* has numerous traditional medicinal applications, for instance, leaves for various internal diseases, prevention of leprosy, dermatoses, as tonics, infusions, diuretics, for stomach ache, cough and so on. A range of phytochemical compounds have been isolated from different plant parts. Many of the uses have been subjected to some level of pharmacological screening, and tests on its antimalarial, anti-diarrhoeal, antibacterial, anti-cough, anti-inflammatory, anti-oxidant activity have been positive. Many of these tests, however, are still at a preliminary level, and need to be followed by more detailed research.

Plate1. *Close view of Guierasenegalensis in its natural habitat*

PHYTOCHEMISTRY

Phytochemicals screening of different parts of *Guierasenegalensis* have been investigated by researchers. They found that leaves revealed the presence of alkaloids, flavonids, tannins [4], saponins [5]and terpenoids/sterols [4]. Also, it reported a new methoxylatednaphthyl butanone; guieranone A was isolated from the leaves of *Guierasenegalensis*. Its structure was elucidated as (2E)-1-(1,3,6,8-tetramethoxy-2-naphthyl) but-2-en-1-one, on the basis of spectroscopic data. Thirteen components were identified for hexane-acetone (50:50) column fraction of hydroacetone extract and twenty-one compounds for hexane-acetone (50:50) column fraction of aqueous decoction extract [6]. Also, Methyldihydroflavasperone, a novel naphthopyran, was isolated from the chloroform extract of the leaf of *Guierasenegalensis*[7]. Mucilagines, tannins, flavonoids, alkaloids and amino acids are the so far known constituents of *Guierasenegalensis*[8]from the methanolic extract of the dried leaves of this plant, flavonolaglycones as well as flavonol glycosides, some of them acylated, were isolated [9]. Four flavonoids from the leaves of *G. senegalensis*, namely catechin, myricitrin, rutin and quarterin have been isolated by [10].

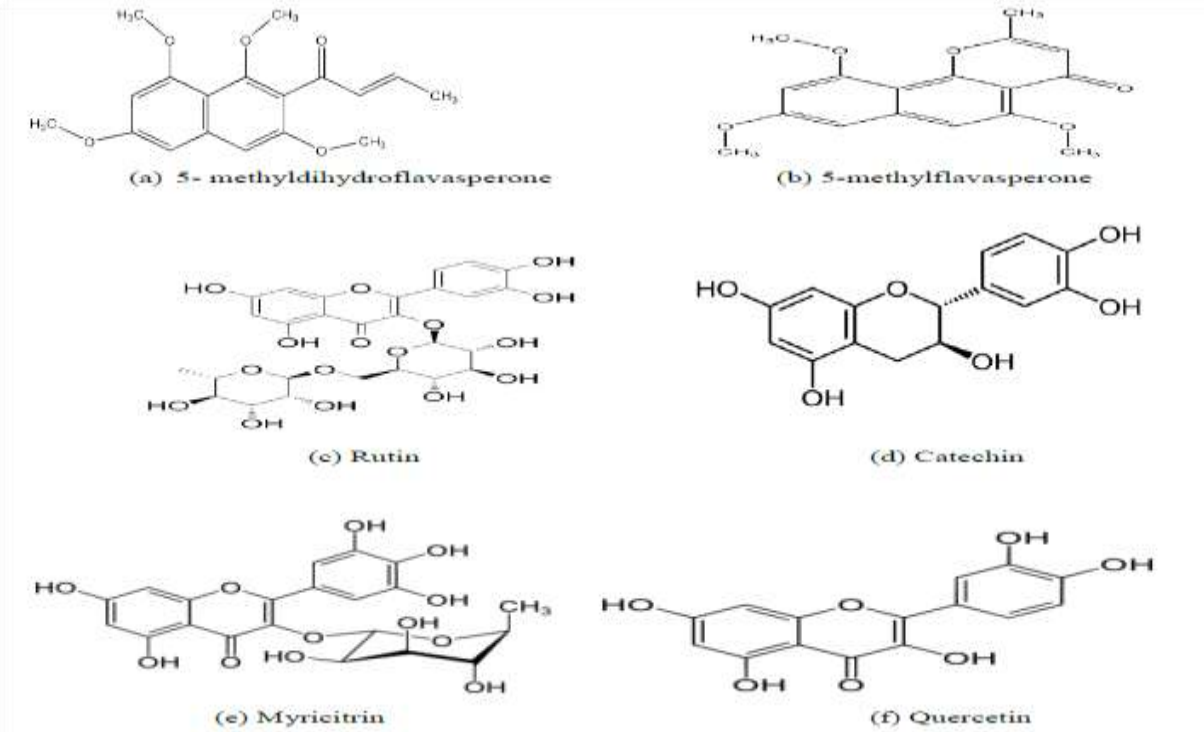

Figure1. *Structures of some compounds isolated from Guierasenegalensis*

METHODOLOGY

Both plant leaves were collected from Federal University Dutse (F.U.D) Jigawa State, Nigeria on May 13th 2018. Below is the table for the coordinates using Global positioning system device (GARMIN GPS 76).

Table1. *Coordinates of Plants Materials (Leaves) Collected*

Plant's Name	Coordinate	Elevation (m)	Accuracy (m)
GuieraSenegalensis	N 11°42.093' E009°22.822'	440.0	20.1

The plant was authenticated by Mal. NamadiSunusi at the herbarium unit in the Department of Plant Science, Ahmadu Bello University, Zaria, Nigeria. Where it was deposited and voucher number01823 was assigned to it. The plant's leaves were chopped into pieces using iron knife, oven dried at 80°C for one (1) hour, pulverised using mortar and pestle, and sieved using a 600 MICS size sieve and then stored in a non-absorptive nylon for subsequent use.

Extraction of Plant Material

The plant material was 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 until complete extraction. The solvents were removed and concentrated using a rotary evaporator and stored in a screw cap bottles at 0^{o}C until usage then labelled.

Preliminary Phytochemical Analysis

The extracts were subjected to various phytochemical tests to identify the constituent secondary metabolites using standard methods [11,12] with some modifications. The metabolites tested for includes: anthraquinones, alkaloids, cardiac glycosides, flavonoids, glycodsides, saponnins, steroids, tannins and terpenes.

TEST FOR STEROIDS/TERPENES

Liebermann-Buchard Test

To each sample (0.5 g) in 20 ml test tube was added chloroform (1 ml) and a few drops of acetic anhydride were added followed by concentrated sulfuric acid. The mixture was carefully mixed and a blue that changed with time was observed in the resulting solution which indicated the presence of steroids /terpenes [13].

Salkowski Test

To each sample (0.5 g) in a 20 ml test tube was added chloroform (1 ml) and to it 1 ml of concentrated sulfuric acid was added down the test tube to form two phases. Formation of yellow coloration was taken as an indication for the presence of sterols [13]

TEST FOR FLAVONOIDS

Shinoda Test

To 1 g sample in 20 ml test tube was added methanol (5 ml). Also, to the sample was added three pieces of magnesium chips followed by few drops of concentrated HCl. A purple colour was observed which indicated the presence of flavonoids [12].

Sulphuric Acid Test

Little quantity of the extract was dissolved in 1 ml concentrated sulphuric acid and a colour changed was observed which indicated the presence of flavonoids [13].

Lead Acetate Test

A small quantity of the extract was dissolved in water and filtered. Few drop of 10 % lead acetate was added to 5 ml of the filtrate. A buff coloured precipitate indicated the presence of flavonoids [14].

Sodium Hydroxide Test

To 1 g sample in 100 ml beaker was added 10% aqueous sodium hydroxide solution (5 ml) and filtered to give yellow colour, a change in colour from yellow to colourless on addition of dilute HCl was observed which indicated the presence of flavonoids [15].

Test for Alkaloids

To each sample (0.5 g) in 20 ml test tube was added 5 ml of 1% aqueous hydrochloric acid then stirred on a water bath and filtered. The filtrate (3 ml) was divided into three. To the first portion, three drops of freshly prepared Dragendoff's reagent was added and an orange to brownish precipitate was observed. To the second portion 1 drop of Mayer's reagent was added and yellowish colour precipitate was observed. To the third portion 1 drop of Wagner's reagent was added to give a reddish-brown precipitate which indicated the presence of alkaloids [13].

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 were formation of green precipitate was observed which indicated the presence of tannins [16].

Test for Anthraquinones (Free Anthraquinones)

Small quantity of the extract was shaken with 10 ml of benzene, the content was filtered, and 5 ml of 10% ammonia solution was added to the filtrate then, the mixture was shaken. No colour change was observed in the ammoniacal layer (Lower phase) which indicated the presence of free anthraquinones [15].

Test for Saponnins (Frothing Test)

About 0.1 g of the extract was shaken with water in a test tube. Frothing was observed which persisted for 1 min that indicated the presence of Saponnins [13].

Test for Glycosides (Ferric chloride Test)

To small quantity of the extract was added 5 ml of conc. H_2SO_4 and boiled for 15 min. This was then cooled and neutralized with 20% KOH. The solution was divided into two portions. Three drops of ferric chloride solution were added to one of the portions, and a green to black precipitate indicated phenolic glycone as a result of hydrolysis of glycoside [13].

Test for Cardiac glycoside (Kella-Killani Test)

To each sample (0.5 g) in 20 ml test tube was added glacial acetic acid (5 ml) containing traces of ferric chloride. The test tube was held at an angle of 45^{o} and concentrated sulphuric acid (1 ml) was added carefully down the side. A purple ring colour at the interface was observed which indicated the presence of cardiac glycoside.

Thin Layer Chromatography Profiling of the Extracts

Thin layer chromatography was carried out on TLC plastic sheet of silica gel pre-coated with layer thickness of 0.2 mm using various solvent system comprising hexane/ethyl acetate mixtures (99, 98, 97, 96, 95, 93, 91, 90, %).

Spotting and development

Spots were applied manually using capillary tube; plates were dried using air blower and developed at room temperature using Shandon chromate tank.

Detection of spots

Spots on TLC plates were visualized using destructive method by spraying with 10% sulphuric acid in methanol, followed by heating at 110^{o}C for about 1 min by holding with a thong in an oven.

RESULTS AND DISCUSSION

Results of extraction, phytochemical screening, free radical scavenging and TLC profiling of the leaves of *Guierasenegalensis*.

DISCUSSION

From the above results, the weight of the crude extracts was found to be13.20; 1.80; 8.40; 7.40; 10.60; (g) for crude extract of hexane, diethyl ether, chloroform, ethyl acetate and methanol extract with hexane having the largest mass (Table 1) using the aforementioned successive soxhlet extraction technique. The preliminary phytochemical screening reveals the presence of anthraquinones (free anthraquinones and combined anthraquinones), alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponnins, steroids/ terpenes, phenolic compounds and tannins, and absence of glycosides (Table 2). This may account for the various uses of the plant in ethno-botanical and traditional medicines. Also, the TLC profiling was carried to know the solvent system that could possibly be used in the further isolation of the crude extract from the plants. Although, an ascending chromatography it gives an idea about the possible compounds that can be present in the extract from the number of spots (Table 3), and also, the possible solvent mixture that could be used for further isolation.

Table2. *Result of Extraction*

Sn	Sample	Weight Used (G)	Solvents Used	Weight Of Crude Extract(G)	% Recovery Yield
1	*GuieraSenegalense*/Leaf	400	Hexane	13.20	3.30
			Diethyl Ether	1.80	0.45
			Chloroform	8.40	2.10
			Ethyl Acetate	7.40	1.85
			Methanol	10.60	2.65

Result of Phytochemical Screening of leaves of *Guierasenegalensis*

Table3. *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	-	+	+	+	+

Result of Thin Layer Chromatography (TLC) Profiling using solvent system comprising hexane/ethyl acetate mixtures (99, 98, 97, 96, 95, 93, 91, 90, %)

Table4. *Thin Layer Chromatography (TLC) Profiling using solvent system comprising hexane/ethyl acetate mixtures (99, 98, 97, 96, 95, 93, 91, 90, %)*

Sno	Solvent	Plant	Spot	Colour 10% H_2so_4
1	Hexane	*GuieraSenegalensis*	1 & 2	Pale green & Bright yellow
2	Diethyl ether	*GuieraSenegalensis*	1 & 2	Green & Pale pink
3	Chloroform	*GuieraSenegalensis*	1,2 & 3	Brown, Deep Blue & Red
4	Ethyl acetate	*GuieraSenegalensis*	1,2,3 & 4	Green, Pale blue, Red & Dark green
5	Methanol	*GuieraSenegalensis*	1,2,3 & 4	Yellow, Blue, Bright green & Black

CONCLUSION

From the above premises it can be concluded that, after the extraction of the crude extracts, the leaves of *Guierasenegalensis* were found to be rich in phytochemicals. Also from relevant literatures, it clearly indicates that, the leaves extracts of *Guierasenegalensis*houses some pure compounds whose structures can be further elucidated and be used for drug design. This means that the leaves would be useful as an antioxidant and free radical scavenging agent and also aids in the treatment of many diseases mediated by reactive oxygen species amongst others.

RECOMMENDATION

From the results obtained in this research, the following recommendations were made;

It is recommended that further research should be carried out to elucidate the structures of bioactive compounds responsible for and also, mechanism of action through which the extract exert the antipyretic and antioxidant activity.

Also, government agencies should educate the populace on the use of medicinal plants which serves as a reservoir for many antioxidants either as vegetables, tea, dietary supplements, etc.

REFERENCES

[1] Ahn, K. (2017). "The worldwide trend of using botanical drugs and strategies for developing global drugs". *BMB Reports*. *50*(3), 111-116. doi:10.5483/BMBRep.2017.50.3.221. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5422022

[2] Smith-Hall, C., Larsen, H.O., &Pouliot, M. (2012). "People, plants and health: a conceptual framework for assessing changes in medicinal plant consumption". *J EthnobiolEthnomed. 8*, 43. doi:10.1186/1746-4269-8-43.

[3] Shettima, Y. A., Tijjani, M. A., Kanumi, Y. (2012). Phytochemical and antidiarrhoeal properties of methanol extract of Guierasenegalensis J.F. Gmel. *International Journal of Pharmacy*,*3*(11).

[4] Somboro, A. A., Patel, K., Diallo, D., Sidibe, L., &Chalchat, J. (2011) An ethnobotanical and phytochemical study of the African medicinal plant *Guierasenegalensis* JF Gmel. J *Med Plants Res 9*, 1651-1639.

[5] Salihu, S. O., & Usman, A. A. (2015). Antimicrobial and phytochemical study of the bioactive fractions of *Guierasenegalensis* from Alasan Tambuwal, Nigeria. *Journal of Pharmacognosy and Phytochemistry 6*, 106-111.

[6] Sombié, P. A., Konate, K., Youl, E., Coulibaly, A. Y., &Kiendrébéogo, M. (2013) GC-MS analysis and antifungal activity from galls of *Guierasenegalensis* JF Gmel (Combretaceae). *Journal of Applied Pharmaceutical Science 12*, 6.

[7] El Hadi, N. M., & Khalid, S. A. (1997). 5-methyldihydroflavasperone, a dihydronaphthopyran from *Guierasengalensis*. *Phytochemistry 4*, 793-794.

[8] Makkar, H. P., & Becker, K. (1994). Isolation of tannins from leaves of some trees and shrubs and their properties. *Journal of Agricultural and Food Chemistry 42*, 731-734.

[9] Bucar, F., Schubert-Zsilavecz, M., &Knauder, E. (1996). Flavonoids of *Guierasenegalensis*. *Pharmazie*, *7*, 517-518.

[10] Ficarra, R., Ficarra, P., Tommasini, S., Carulli, M., &Melardi, S. (1997). Isolation and characterization of *Guierasenegalensis* JF Gmel. Active principles. *BollettinoChimico Farmaceutico 5*, 454-459.

[11] Harbone, J. B. (1973). Phytochemical Methods: A guide to modern technique of plant Analysis. *Chapinan and Hall*, (pp. 33-185). London.

[12] Sofowara, A. (1993). Medicinal plants and Traditional medicine in Africa. Ibadan, Nigeria: *Spectrum Book LTD*. (pp. 289).

[13] Silva, G.L., Lee, I., & Douglas, K.A., (1998). Special Problems with Extraction of Plants: Cannel, J.P.R. (ed.). Natural Products Isolation. Humana press publishers, (pp 356-358). New Jersey (USA).

[14] Brain, K. R., & Turner, T. D. (1975). *The practical evaluation of phytopharmaceuticals* (Vol. 1). Bristol: Wright-Scientechnica.

[15] Cannel, R. J. P., (2000). Methods in Biotechnology.Natural Products Isolation. *Human pres*, (pp. 14-36). New Jersey.

[16] Trease, G. E., & Evans, W. C. (1996). Textbook of Pharmacognosy, 12th (Ed) Balliers Tindall Publisher Limited, (pp. 343-384). London, United Kingdom.

Citation: *Lawal A.M., Lawan M. M., Apampa S. A, "Phytochemical Analysis and Thin Layer Chromatography Profiling of Crude Extracts from GuieraSenegalensis (Leaves)". Open Access Journal of Chemistry, 3(2), 2019, pp.7-12*