

Phytochemical Screening and Antibacterial Activity of Leaf Extracts of *Guiera senegalensis* and *Eucalyptus camaldulensis*

*¹Bello, A. A., ²Zainab, A. Z., ²Muhammad U. A.

¹Department of Biological Sciences,
Fika Government Secondary School Potiskum,
Yobe State, Nigeria.

²Department of Biological Sciences,
Faculty of Science,
Bauchi State University
Gadua.

Email: abdullahihabubello@gmail.com

Abstract

Guiera senegalensis and *Eucalyptus camaldulensis* are multipurpose trees cultivated in several tropical and subtropical regions of West and North Africa for their medicinal values. Therefore, this study was aimed to investigate the phytochemical compositions and antibacterial activity of the ethanol and aqueous extracts of *Guiera senegalensis* and *Eucalyptus camaldulensis* against *Salmonella typhi*, *Salmonella paratyphi A*, and *Salmonella Paratyphi B* through disc diffusion method. The ethanol and aqueous extract of *Guiera senegalensis* and *Eucalyptus camaldulensis* was prepared through maceration method. The extracts and Ciprofloxacin were prepared at different concentrations (25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml). Phytochemical investigation revealed the present of Flavonoids, Saponins, Steroids, Terpenes, Tannins and Cardiac glycosides in the extracts both plants. The ethanol extract of the screened plants proved to be more sensitive against all test organisms which showed a significant zones of inhibitions ranging between (8.00±0.508 – 13.67±0.50mg/ml) compared to the aqueous extracts which had zones of inhibitions ranging from (7.00±0.50 - 10.00±0.50mg/ml). In-vitro antibacterial activity of all the plant extracts indicates the MIC and MBC ranging between (25 – 100mg/ml) and (50 – 200mg/ml) respectively, but there was no turbidity observed for Ciprofloxacin discs. The screened plant extracts were found to have bioactive potentiality which is effective against all the test organisms. Therefore, this result may support the use of these plant parts in herbal medicine to treat typhoid fever.

Keywords: Plant extracts, *Guiera senegalensis*, bactericide, Bacteriostatic and herbal medicine

INTRODUCTION

Guiera senegalensis (Combretaceae) is widely distributed in West African Savannah and grows as a shrub (Sanogo, 2012). is used to treat a wide variety of diseases and the plants are often used combined with other plants to treat common colds, fever and respiratory problems (Sanogo, 2012; Ogbeba, 2017). A range of phytochemical compounds have been

*Author for Correspondence

isolated from different plant parts such as tannins, flavonoids, alkaloids and mucilage Fiot *et al.* (2006). It has been subjected to some pharmacological screening on antimalarial, anti-diarrhoeal, antibacterial, anti-cough, anti-inflammatory, anti-oxidant activity with positive effect Fiot *et al.* (2006). The *Eucalyptus camaldulensis* is commonly known as River Red Gum, it is a native to Australia grown plantation species in many parts of the world Babayi *et al.* (2004). *Eucalyptus camaldulensis* extract has many properties such as anti-cancer, anti-inflammatory, painkiller, antioxidant, anti-blood proliferation, anti-malaria, anti-mold and antiviral (Adebola *et al.*, 2000; Siddiqui and Sultan, 2004; Takasaki *et al.*, 2009). Somda *et al.* (2007) Showed that oil extracts of *Eucalyptus camaldulensis* were effective as anti-fungal agents at high concentrations. Adeniyi, *et al.* (2009) showed the *in vitro* susceptibility of *Helicobacter pylori* to extracts of *Eucalyptus camaldulensis* Adeniyi, *et al.* (2009). Many studies have shown that ethnomedicinal plants are the oldest source of bioactive products with proven efficacy Almeida *et al.* (2001) and are the basic components of several pharmacological classes of drugs such as analgesics, anesthetics, and antibiotics, anticancer and diuretic drugs Hemalta *et al.* (2013).

The *Guiera senegalensis* and *Eucalyptus camaldulensis* leaf extracts have been used for the treatment of many diseases by traditional medical practitioners in Nigeria. Therefore, the investigation of these indigenous plants for their antibacterial potentiality will be of utmost importance.

This study was aimed to evaluate the phytochemical properties and antibacterial activity of the ethanol and aqueous extract of *Guiera senegalensis* and *Eucalyptus camaldulensis* for the treatment of typhoid fever, gastroenteritis and diarrhea.

MATERIALS AND METHODS

Collection and Preparation of Plant Samples

The leaves of the trees were collected from Potiskum Local Government Area of Yobe State Nigeria, which was located on A3 highway between latitude 43°22'N and 11°30'N and longitude 3°30'E and 7°20'E with an area of (216sqmi) with a population of 205,876 according to 2006 census, immediately taken for authentication {*Guiera senegalensis* (BUKHAN0032) and *Eucalyptus camaldulensis* (BUKHAN0347)} by taxonomist at the Laboratory of Plant Biology Department, Bayero University, Kano Nigeria. The leaves were dried at room temperature for three weeks and then ground into powdery form using mortar and pestle.

Preparation of the Extracts

One hundred gram of each plant part powder were weighed and percolated separately with 1000ml of respective solvents (70% Ethanol and Water) and kept at room temperature for about seven days with regular shaking. The percolates were filtered through whatman filter paper No. 1 and afterwards the filtrates were evaporated to dryness to obtain the extracts. The extracts were stored in sterilized sample bottles at 15°C until required for further use.

Phytochemical Screening of the Plant Extract

The methods used by [Soforowa, 1993; Gurinder *et al.*, 2009] were employed for phytochemical analysis in order to identify some secondary metabolites such as tannins, alkaloids, flavonoids, glycosides, steroids, Terpens, Saponins, and anthraquinones.

Plant Extract Activity Bioassay

Disc diffusion method of Gamal and Sherbiny, (2015) was employed. The freshly prepared nutrient agar plates were inoculated with 0.1ml of the test organisms by streaking method. The discs were prepared by impregnating each with the ethanol and aqueous extracts of *Guiera senegalensis* and *Eucalyptus camaldulensis* respectively at varying concentrations (25mg/ml 50mg/ml 100mg/ml and 200mg/ml) using sterilize forceps. Four impregnated discs were inoculated onto a prepared nutrient agar plate and incubated for 24 hours at 37°C. The same procedures were carried out using ciprofloxacin as a positive control. The zones of inhibition were measured with the aid of a meter ruler in millimeters and values were tabulated. The antibacterial activities were tested against the clinical strains of *Salmonella typhi*, followed by *Salmonella paratyphi A*, and then *Salmonella paratyphi B* which were obtained from Aminu Kano Teaching Hospital (AKTH), Kano State, Nigeria. All the clinical strains were identified by using biochemical test in accordance with the method of Cheesbrought (2000) before used. The entire tests were performed in triplicate.

Determination of minimum inhibitory concentrations of the extracts

The method of Gamal and Sherbiny, (2015) was employed with a little modification in which the extract concentrations of 6.25mg/ml using ten-fold serial dilution with distilled water were prepared. Zero point three (0.3ml) was inoculated into nutrient broth tubes at different extract concentrations (3.125mg/ml, 6.25mg/ml, 12.5mg/ml, 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml) and the tubes were incubated at 37°C for 24hrs. The least concentration of the extract showing no visible growth of bacteria was noted and recorded as the MIC.

Determination of minimum bactericidal concentrations of the extract

The concentrations of the plant extracts [3.125mg/ml, 6.25mg/ml, 12.5mg/ml, 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml] were prepared. The minimum bactericidal concentrations were determined from broth dilution test resulting from the MIC tube by sub-culturing to antibacterial free agar as described for the MIC earlier. The lowest concentration of the agent which shows no visible signs of growth was recorded as the minimum bactericidal concentrations Cohen *et al.* (1998).

Statistical Analysis

The values of the zones of inhibition of the *Guiera senegalensis* and *Eucalyptus camaldulensis* leaf extract and ciprofloxacin were expressed as the Mean±SD of three replicates.

RESULTS

Phytochemical screening of the Ethanol and Aqueous Extracts of *Guiera senegalensis* and *Eucalyptus camaldulensis*

The results of the phytochemical analysis of the *Guiera senegalensis* and *Eucalyptus camaldulensis* leaf extracts showed the presence of Flavonoids, Cardiac glycoside, Saponins, Terpenes, Steroids and Tannins but the Anthraquinones were absent in both the plant extracts where as alkaloids were absent only in aqueous extract of *Eucalyptus camaldulensis* as shown in Table 1.

Phytochemical Screening and Antibacterial Activity of Leaf Extracts of *Guiera senegalensis* and *Eucalyptus camaldulensis*

Table 1: Phytochemical constituents of *Guiera senegalensis* and *Eucalyptus camaldulensis* extract

Constitutes	Solvents	<i>Guiera senegalensis</i>	<i>Eucalyptus camaldulensis</i>
Flavonoids	Ethanol	+	+
	Aqueous extract	+	+
Alkaloids	Ethanol	+	-
	Aqueous extract	+	+
Cardiac glycosides	Ethanol	+	+
	Aqueous extract	+	+
Saponins	Ethanol	+	+
	Aqueous extract	+	+
Tannins	Ethanol	+	+
	Aqueous extract	+	+
Anthraquinines	Ethanol	-	-
	Aqueous extract	-	-
Terpenes	Ethanol	+	+
	Aqueous extract	+	+

Key: + is positive, - is negative

Antibacterial activity of *Guiera senegalensis* leave extracts on some Bacteria

The antibacterial activity of the ethanol extract of *Guiera senegalensis* showed a broad antibacterial spectrum against all the test organisms with zone of inhibitions range between (13.67±0.50 - 8.00±0.50mm) compared to the aqueous extract which range between (10.00±0.50 - 8.00±0.50mm) whereas Ciprofloxacin demonstrated the broadest antibacterial spectrum against the all the tested organisms (30 - 23mm) as shown in Table 2.

The result of the zone of inhibitions of ethanol and aqueous extracts of *Eucalyptus camaldulensis* against all test organisms are (13.67±0.50 - 8.00±0.50mm) and (10.00±0.50 - 8.00±0.50mm) respectively against *Salmonella typhi*, *Salmonella paratyphi* A and *Salmonella paratyphi* B whereas Ciprofloxacin was range between (30 - 23mm) as shown in Table 4.

Phytochemical Screening and Antibacterial Activity of Leaf Extracts of *Guiera senegalensis* and *Eucalyptus camaldulensis*

Table 2: Antibacterial activity of *Guiera senegalensis* and *Eucalyptus camaldulensis* leaf extracts on *Salmonella*

Concentration (mg/ml)	Ethanol extract	Aqueous extract	Ciprofloxacin (mg/ml)
<i>Guiera senegalensis</i>			
<i>Salmonella typhi</i>			
25	10.00±0.67	8.00±0.50	26.00±0.50
50	11.00±0.50	8.33±0.50	28.00±0.50
100	12.00±0.50	9.67±0.50	29.00±0.50
200	13.67±0.50	10.00±0.50	30.00±0.50
<i>Salmonella paratyphi A</i>			
25	9.00±0.50	8.00±0.50	24.00±0.50
50	9.67±0.50	9.00±0.50	27.00±0.50
100	10.00±0.50	9.33±0.50	27.00±0.50
200	11.00±0.50	9.67±0.50	29.00±0.50
<i>Salmonella paratyphi B</i>			
25	8.00±0.50	8.00±0.50	23.00±0.50
50	9.00±0.50	8.33±0.50	25.00±0.50
100	9.67±0.50	9.00±0.50	26.00±0.50
200	10.00±0.50	9.67±0.50	28.00±0.50
<i>Eucalyptus camaldulensis</i>			
<i>Salmonella typhi</i>			
25	10.00±0.67	7.00±0.50	26.00±0.50
50	11.00±0.50	8.00±0.50	28.00±0.50
100	11.67±0.50	8.67±0.50	29.00±0.50
200	12.00±0.50	9.00±0.50	30.00±0.50
<i>Salmonella paratyphi A</i>			
25	9.00±0.50	8.00±0.50	24.00±0.50
50	9.67±0.50	8.00±0.50	27.00±0.50
100	10.00±0.50	8.33±0.50	27.00±0.50
200	11.00±0.50	8.67±0.50	29.00±0.50
<i>Salmonella paratyphi B</i>			
25	8.33±0.50	8.00±0.50	23.00±0.50
50	9.00±0.50	8.33±0.50	25.00±0.50
100	9.33±0.50	9.00±0.50	26.00±0.50
200	9.67±0.50	9.67±0.50	28.00±0.50

The results are expressed as Mean ± Standard Error of Mean

Minimum Inhibitory and Bactericidal Concentrations of *Guiera senegalensis* leaf extract of Bacteria

The result of the minimum inhibitory concentration (MIC) of the two different extracts on all test organisms. The MIC of *Salmonella typhi* was 25mg/ml for the ethanol extract but 12.5mg/ml for the aqueous extract whereas minimum bactericide concentration (MBC) of the ethanol extract was 100mg/ml and 25 for the aqueous extract. *Salmonella paratyphi A* and *Salmonella paratyphi B* were found to have 100mg/ml for the ethanol extract and 200mg/ml for the aqueous extract respectively but there was no turbidity observed in the minimum inhibitory concentration of Ciprofloxacin against all the tested organisms Table 3.

Table 3: Minimum Inhibitory and Bactericidal Concentrations of *Guiera senegalensis* extract of Bacteria

Test Organism	Ethanol extract		Aqueous extract	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
<i>Salmonella typhi</i>	25	100	12.5	25
<i>Salmonella parayphi A</i>	100	200	100	200
<i>Salmonella parayphi B</i>	100	200	100	200

MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericide Concentration

Minimum Inhibitory and Bactericidal Concentrations of *Eucalyptus camaldulensis* leave extract of Bacteria

Table 4 showed minimum inhibitory concentration (MIC) of the two different extracts on all test organisms. The MIC of *Salmonella typhi* was 50mg/ml for the ethanol extract but 6.25mg/ml for the aqueous extract whereas minimum bactericide concentration (MBC) of the ethanol extract was 100mg/ml and 12.5 for the aqueous extract. *Salmonella parayphi A* and *Salmonella parayphi B* were found to have 100mg/ml for the ethanol extract and 200mg/ml for the aqueous extract respectively but there was no turbidity observed in the minimum inhibitory concentration of the Standard antibiotic (Ciprofloxacin) against all the tested organisms, this implies there was no growth by the tested organism Table 4.

Table 4: Minimum Inhibitory and Bactericidal Concentrations of *Eucalyptus camaldulensis* extract of Bacteria

Test Organism	Ethanol extract		Aqueous extract	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
<i>Salmonella Typhi</i>	50	100	6.25	12.5
<i>Salmonella parayphi A</i>	100	200	100	200
<i>Salmonella parayphi B</i>	100	200	100	200

MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericide Concentration

DISCUSSION

The result of the phytochemical analysis of the extracts of *Guiera senegalensis* revealed the presence of Alkaloids, Flavonoids, Saponins, Tannins, Terpenes, Cardiac glycosides and Steroids while the Anthraquinones were absent. This study corroborated with the previous finding of Fiot *et al.* (2006). Who carried similar worked on the phytochemical study on the roots and leaf of *Guiera senegalensis*. The *Eucalyptus camaldulensis* consist of Flavonoids, Saponins, Tannins, Terpenes, Cardiac glycosides and Steroids while the Alkaloids and Anthraquinones were absent. This study corresponded with the previous finding of (Aliyu, 2006). who reported that *Eucalyptus camaldulensis* leaf had many secondary metabolites which are bioactive against ingredients.

In this study, the result of the antimicrobial activities obtained indicates that the leaf extracts of *Guiera senegalensis* and *Eucalyptus camaldulensis* at different concentrations were quite effective against all the tested organisms. This showed that the extract of *Guiera senegalensis*

contains the substances that can inhibit the growth of all the tested organisms. This is in line with the findings of Ogbeba *et al.* (2017) who reported that *Guiera senegalensis* leaf extract inhibited the growth of *Salmonella species* at different concentrations. This might be due to the presence of some secondary metabolites in the screened plant leaves Aniagus *et al.* (2005). The result also indicated that almost all the concentration of the *Eucalyptus camaldulensis* leaf extracts used was effective against all the tested organisms. This study corroborated with the previous findings of (Adeniyi, 2009) who similarly worked on the efficacy of *Eucalyptus camaldulensis* on salmonella species and attributed the action of the plant to this bioactive properties.

The antibacterial pattern in this study indicated that ethanol extract had the highest zones of inhibition compared to the aqueous extract. This study agreed with the previous findings of Ogbeba *et al.* (2017) who carried similar work on plant extract against *Salmonella typhi*. This is because the ingredients dissolved better in ethanol solvent than aqueous solvents. Result of the minimum inhibitory concentration of the extracts revealed the broad spectrum antibacterial activity at concentration of 3.125mg/ml against all the test organisms. This implies that the extract showed bactericidal activity against *Salmonella typhi*, *Salmonella paratyphi A*, and *Salmonella paratyphi B*. This study corroborated with the previous finding of (Amenu, 2014).

CONCLUSION

This study revealed that the leaves of *Guiera senegalensis* and *Eucalyptus camaldulensis* possess antibacterial properties; this is due to the presence of some secondary metabolites in the plant's leaves. The *Guiera senegalensis* leaf extract possess higher antimicrobial activities than the *Eucalyptus camaldulensis* leaf extracts. The ethanol extract are more active than aqueous extract. A further investigation on *in-vivo* antimicrobial activity on the leaves extract of *Guiera senegalensis* and *Eucalyptus camaldulensis* is recommended in order to draw a solid conclusion.

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