PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF GAMBIA BUSH TEA (*Lippia multiflora)* AND *Combretum glutinosum* PLANTS USED AS MEDICINAL PLANTS IN THE GAMBIA.

IYEKOWA, O.[1]*, FAAL, F.[2] AND OYELAKIN, O.[2]
[1]Department of Chemistry, University of Benin, Benin City, Nigeria.
[2]Chemistry Unit, Division of Physical and Natural Sciences, School of Arts and Sciences, University of The Gambia.

ABSTRACT

Medicinal plants in The Gambia and most West African countries are generally sold in the markets especially in rural areas and the rate at which they are employed by users for the treatment of various ailment without recourse to knowledge of the chemical constituents, safety and dosage is worrisome. In this research, a preliminary investigation was conducted to assess the phytochemical constituents and antimicrobial activity of *Lippia multiflora* and *Combretum glutinosum* used as poultice for skin infections in The Gambia. Extraction was conducted using Soxhlet extractor with methanol as a solvent while phytochemical screening was performed according to standard methods. The micro-organisms used for the antimicrobial assay were six clinical pathogens; *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis* and *Streptococcus pneumonia.* The result of the phytochemical screening showed that the methanol extract of *L. multiflora* contains alkaloids, saponins, flavonoids, tannins, glycosides, phenolics, terpenoids and eugenols, while *C. glutinosum* contains glycosides, saponins, flavonoids, eugnols, terpenoids, and alkaloids. The antimicrobial analysis revealed zones of inhibition at a concentration of 1000 mg/mL: 05.40 mm (*Echerichia coli* ATCC 25922), 27.50 mm (*Staphylococcus aureus ATCC* 2592317), 15.40 mm (*Pseudomonas aeruginosa* ATCC 27853), 05.00 mm (*Streptococcus pneumonia ATCC 49619*) and 21.50 mm (*staphylococcus epidermidis ATCC* 12228) for *L. multiflora* methanol extracts while *C. glutinosum* indicated zones of inhibition at same concentration with: 0.50 mm (*Echerichia coli* ATCC 25922), 32.50 mm (*Staphylococcus aureus ATCC* 2592317), 5.50 mm (*Pseudomonas aeruginosa* ATCC 27853), 25.00 mm (*Streptococcus pneumonia ATCC 49619*) and 26.50 mm (*Staphylococcus epidermidis ATCC* 12228). Both plant extracts indicated activity when compared with the standard control (Ciprofloxacin). The result of this study showed that the two plants contained phytochemical constituents and possesses antimicrobial activity which corroborate the use of the plant as supplementary sources for the traditional treatment of skin infections caused by microorganisms.

Keywords: antimicrobial activity, *Combretum glutinosum, Lippia multiflora*, phytochemical
***Correspondence:** osaro.iyekowa@uniben.edu. +2348056733021

INTRODUCTION

Medicinal plants have been known to play a vital role in human sustenance. They give nutrient through edible parts and bioactive ingredients as medicine for our health through phytochemicals. Medicinal plants have been used to treat infectious diseases for many years worldwide leading to a growing interest in the development of drugs from these plant species [1]. The Gambia, among other African countries, is one of the countries in the world with unique wealth of medicinal plants and vast traditional knowledge of use of herbal medicine for treatment of various ailments [2]. The important role of medicinal plants cannot be overstressed since they lead to the isolation of many important drugs of modern day. Almost 80% of the human populations in developing countries are dependent on plant resources for their primary healthcare [3].

Lippia multiflora (family, *Verbenaceae*) is one of the major medicinal plants used traditionally for treatment of diseases ranging from dysentery, stomach ailments, skin and venereal infections in The Gambia. *L. multiflora* is an aromatic shrub that can grow to a height of 2.7 m to 4.0 m. The elliptic leaves are up to 11cm long and grey beneath, with a toothed margin and arranged in whorls [4]. The leaves have a stronger camphor odour at bruising and the apex is very obtuse [5]. The distribution range of *L. multiflora* has its major concentrations in Guinea Savannah, forest Savannah and Transitional and Coastal Savannah zones [6]. This plant is commonly known as *Lippia* tea and commercially known as "Gambian Tea Bush" "Bush Tea", and "Healer Herb" [7]. *L. multiflora* is locally known as "Mbormbor" in Wolof, "Sisilinyamo" in Mandinka and "Baye baye" in Fula. It is widely spread in Central River and Upper River regions in The Gambia.

The *Lippia* species have a long history of traditional medicinal application, some have scientific validation. They are mostly used in the treatment of skin, respiratory and gastrointestinal disorders. They exhibit anti-malarial, spasmolytic, sedative, hypotensive and anti-inflammatory activities [8]. It is also to treat

bronchial inflammation, conjunctivitis, gastro-intestinal disturbance, poultice for skin infection, enteritis, coughs and colds [8], and possesses hypotensive, fatigue relieving, and diuretic properties [5]. Some rural dwellers cook the herbs and use it to relieve stress and enhance sleep, also as a substitute for tea and as a mouth disinfectant [7]. In Nigeria, the leaves are used for constipation and as antipyretic while in The Gambia *L. multiflora* is pounded and apply on one's head for lice. It is then rinsed off with hot water the next day [9]. *L. multiflora* is among the more recommended and sold plants for the treatment of diabetes [10]. Despite its importance, the species is still less researched and the aspects studied are only related to the characteristics of its essential oil [11]. The essential oil isolated from *L. Multiflora* also contains high content of terpenoids among which included linanool, myrcene, limonene and beta-farnesene [12]. Recent studies of phytochemistry of *L. multiflora* revealed the presence of alkaloids, tannins, terpenoids and flavonoids in the leaves while steroids and quinones were detected in the flower [13].

Combretum glutinosum (family *Combretaceae*) has also been reported to be of great importance in traditional medicine. *C. glutinosum* is a shrub or small tree that can grow up to 12-15 m tall. It is 60 cm in diameter, often crooked or branched from the base. The bark is grey, rough and grooved. *C. glutinosum* is locally known as "rat" in Wolof, "Jambakatang" in Mandinka, "Kantakara" in Hausa and "doki" in Fula. It is widely spread in Central River and Upper River regions in The Gambia.

In West Africa the leaves, stems and root bark of *C. glutinosum* are important sources of yellow to brownish yellow dyes for cotton textiles, leather and mats of various fibers. In The Gambia the twigs of *C. glutinosum* are used as chewing sticks to prevent toothache [14], and leaves for tea generally for a variety of ailments including those of lungs, joints, teeth and skin. The wood is yellowish, hard and very durable and is used in house construction, for tool handles and as firewood. A decoction or infusion of the leaves, bark or fruits is very popular, mainly to treat urinary, liver and kidney complaints, but also all kinds of respiratory problems, fevers, intestinal complaints, cold, headache, chest pain and to clean wounds and sores on any part of the skin [15].In traditional veterinary medicine, the dry powdered leaves and bark are used to dress wounds. In Senegal the gum of the bark is used to fill the cavity of a carious tooth. Young shoots and roots are believed to have aphrodisiac properties [15].

Pharmacological studies and biological activity carried out by various workers have shown that the plants have several active compounds, which justify the plant's medicinal use. Studies have shown that *C. glutinosum* is used in the treatment of snake bites, scorpions' stings, infertility in women, pneumonia, cold, weak muscles, constipation, cough, headache, jaundice, liver illness, pneumonia, chest pain and swelling caused by mumps [14]. The methanol extracts of C. *glutinosum* showed anti hepatitis B activity in *in-vitro* and contain gallic tannins. The extracts also have shown activity on the reversibility of the sickling of sickle cells [16]. Among many phytochemicals, flavonoids and terpenoids have been isolated from the leaves of *C. glutinosum* by Dawe [17].

Recently there have been reported cases of opportunistic infections, toxicity of many antimicrobial drugs and this has imposed pressure on the scientific community and pharmaceutical companies to search alternative and novel drug sources. Thus, one of the strategies in the search for new antimicrobial agent is a study of active constituents of medicinal plants. The study is aimed at the preliminary screening of the two plants for its phytochemical constituents and antimicrobial activities using selected pathogens.

Plate 1: *Lippia multiflora*

Plate 2: *Combretum glutinosum*

MATERIALS AND METHODS

Sample collection and treatment

The leaves of *L multiflora* and *C. glutinosum* were collected fresh from Faal's Kunda (compound) in Northwest District of The Gambia. Both plants were identified and authenticated where voucher specimen numbers: UBHm 01907 and UBHm 01908 were respectively deposited in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The leaves were washed with distilled water, air-dried under shade in the laboratory for four weeks and pulverized to a powdered form. One hundred and fifty grams (150 g) of the powdered plants were separately extracted with 500 mL of methanol solvent for an 8-hour in a Soxhlet extractor. Both extracts were

concentrated separately using a rotary evaporator (Model, RE 200).

Phytochemical screening
Phytochemical screening was done to identify the presence of chemical constituents such as alkaloids, glycosides, steroids, flavonoids, saponins, terpenoids, phenolics, and eugenols by using methods described by Sofowora [18] and Trease and Evans [19].

Microorganisms
The microorganisms employed in this study were obtained from the Clinical Microbiology Laboratory, Medical Research Council at London School of Hygiene and Tropical Medicine (MRC, LSHTM), The Gambia which includes clinical isolates of *Escherichia coli* ATCC 25922*, Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus pneumonia* ATCC 49619 and *Staphylococcus epidermidis* ATCC 12228.

Media
Muller Hinton agar, nutrient broth, and Blood agar, all product of Himedia Laboratories Mumbai (India) were used in this study. The composition of the medium was beef extract 3.0 g, peptone 5.0 g, Sodium chloride 8.0 g and agar 15.0 g.

Agar well diffusion assay
The antimicrobial activity of the extracts was determined by using the agar well diffusion technique by Monica [20]. Nutrient agar plates were each seeded with 0.1ml of an overnight culture of each bacterial (10^6 CFU/mL). The 24 hours broth culture of each bacterium were used to seed sterile molten nutrient agar at 45^0C, allowed to set and well made by sterile standard cork borer (6.0 mm in diameter) and varying concentration of both extracts added into each well separately. The bacterial plates were incubated at 37^oC for 24 hours, after which diameter of zones of inhibition were measured for the two plant extracts [20]

Determination of minimum inhibitory concentration (MIC)
The MIC values of each plant extracts were determined using two fold micro-dilution to prepare concentrations of 1000 mg/mL (by dissolving 1g of dried extract in 1ml of normal saline), 500 mg/ml, 250 mg/mL, 125 mg/mL of each extract and a drop of the bacterial suspension that had been previously diluted to about 10^6 CFU/mL were aseptically incorporated into molten nutrient agar and allowed to set. The plates were incubated at 37^0C for 24 hours. The lowest concentration preventing visible growth for each of the test organisms was recorded as the MIC. The experiments were carried out in triplicate for each extract concentration and ciprofloxacin as positive control.

RESULTS

Phytochemical screening
The results of the phytochemical screening of both plants extracts are shown in Table 1 below.

Table 1: Phytochemical result of *L. multiflora* and *C. glutinosum*

S/N	Phytochemicals	*L. multiflora*	*C. glutinosum*
1	Glycosides	+	+
2	Steroids	-	-
3	Terpenoids	+	+
4	Alkaloids	-	-
5	Saponins	+	+
6	Flavonoids	+	+
7	Tannins	+	-
8	Phenolics	+	_
9	Eugenols	-	-

Key: Present = + Absent = -

Antimicrobial activity results
Table 2: Zones of inhibition of *L. multiflora* against some selected pathogens

Microorganisms	Minimum inhibitory concentration (MIC) (mg/mL)				
	1000 mg/mL	500 mg/mL	250 mg/mL	125 mg/mL	Ciprofloxacin 0.01 mg/mL
	Zone of inhibition (mm)				
***E. coli* ATCC 25922**	5.40	5.00	0.00	0.00	36.00
***S. aureus* ATCC 25923**	27.50	25.00	16.00	27.00	36.00
***P. aeruginosa* ATCC 27853**	15.40	10.50	10.00	10.00	36.00
***S. pneumonia* ATCC 49619**	5.00	4.00	4.00	0.00	36.00
***S. epidermidis* ATCC 12228**	21.50	20.00	20.00	16.00	36.00

Key: ATCC = American Type Culture Collection. (-) – no activity < 10 mm - non significant activity; 10-19 mm – significant activity > 20 mm – high activity (National committee for clinical laboratory standard [21]).

Table 3: Zones of inhibition of *C. glutinosum* against some selected pathogens

Microorganisms	Minimum inhibitory concentration (MIC) (mg/mL)				
	1000 mg/mL	500 mg/mL	250 mg/mL	125 mg/mL	Ciprofloxacin 0.01 mg/mL
	Zone of inhibition (mm)				
***E. coli* ATCC 25922**	0.50	0.00	0.00	0.00	36.00
***S. aureus* ATCC 25923**	32.5	32.00	30.00	27.00	36.00
***P. aeruginosa* ATCC 27853**	5.50	0.50	0.00	0.00	36.00
***S. pneumonia* ATCC 49619**	25.00	24.00	16.00	10.5	36.00
***S. epidermidis* ATCC 12228**	26.5	20.00	12.50	11.70	36.00

Key: ATCC = American Type Culture Collection. (-) – no activity < 10 mm - non significant activity; 10-19 mm – significant activity > 20 mm – high activity (National committee for clinical laboratory standard [21])

DISCUSSION

In Table 1, glycosides, saponins, alkaloids, terpenoids and flavonoids were indicated among others in methanol extract of both *L. multiflora* and *C. glutinosum.* These phytochemicals are useful bioactive agents that have physiological effect in man [18]. Different phytochemicals have been found to possess a wide range of activities which may help in protection against chronic diseases. For example, alkaloids are used in relieving pains, anxiety and depression, they are important drug source reported to possess antimicrobial, antioxidant and cytotoxic activity [22].

The antimicrobial activity of aqueous extract of *L. multiflora* (Table 2) displayed high zones of inhibition at concentration of 1000 mg/mL against S. aureus (27.50 mm) and *S. epidermidis* (21.50) when compared with the standard antibiotic (ciprofloxacin). The acceptable standard zones of inhibition for sensitive organism for the antibiotic ciprofloxacin is greater than 21 mm (National committee for clinical laboratory standard [21]. For *C. glutinosum,* at 1000 mg/mL, zones of inhibition recorded were: *S. aureus* (32.50 mm), *S. pneumonia* (25.00 mm) and *S. epidermidis* (26.50 mm) (Table 3). Low zones of inhibition were observed for both plants with *E. coli*. However, the studies of Owolabi *et al.* [23] indicated no antibacterial activity with the hexane extract of *L. multiflora* against *Bacillus cereus* and *Staphylococcus aureus*. This result is suggestive of the fact that many bioactive constituents are extracted by polar solvents like methanol. In this study, both plants displayed dose dependent activity. Bassole *et al.* [24], conducted a research and reported that *L. multiflora* essential oil had the highest inhibitory activity against *E. coli* and this research indicated low inhibitory activity against *E. coli* which suggest that non polar fractions containing essential oils has activity against *E. coli* than polar fractions as used in this study. From the work of Ousmane [25], methanol extract gave the highest zones of inhibition among other extracts used in his work against *S. aureus*. This research also shows similar activity for *S. aureus.* More so, the methanol extract of the stem bark of *Combretum glutinosum* as investigated by Usman [26] showed the highest level of inhibition on *Salmonella typhi* and *Escherichia coli*, an indication of the presence of bioactive constituents also in the stem.

CONCLUSION

The results of the study showed that *Lippia multiflora* contained phytochemical substances with antimicrobial activity especially against skin infections which could arise from *Staphylococcus epidermidis* and *Staphylococcus aureus* while *Combretum glutinosum* indicated activity against *Streptococcus pneumonia, Staphylococcus epidermidis* and *Staphylococcus aureus.* The findings of this research indicated that extracts of both plants when isolated and characterized will be a promising antimicrobial agent.

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