Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(6): 945-948
Received: 04-09-2017
Accepted: 05-10-2017

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Comparative Phytochemistry and *In Vitro* Antimicrobial Effectiveness of the Leaf Extracts of *Clerodendrum capitatum* (Verbenaceae) and *Ficus glumosa* (Moraceae)

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Abstract
Plant as drug has been used since the period of Neanderthals as well as the time of primitive man across the World. Infectious diseases are among the most causes of death particularly in poorly hygienic areas in most developing countries like Nigeria. Two plant species of different families were used in this study to compare the choice as well as their usage against some infectious in Northern Nigeria. The leaf sample of *Clerondendrum capitatum* and *Ficus glumosa* were separately collected from Demsa Local Government Area, and Nkafimiya village, Michika Local Government Area, Adamawa State, The air-dried powered plant materials of *C. capitatum* and *F. glumosa* (250 g) each were extracted for 4 hours with 70% ethanol using reflux apparatus; the concentrated extracts were air-dried under reduced pressure and temperature and then subjected to phytochemical evaluation and subsequent antimicrobial activities using hole-in-plate disc diffusion technique was ascertained on some pathogenic organisms, three of which were Gram positive, three Gram negative and a fungal species. The susceptibility data at the highest dose (80 mg/hole) revealed that *Clerodendrum capitatum* was highly effective on *Candida albicans* with the value of 20.33±0.58 mm while it was least active on *Staphylococcus aureus* (7.33±0.58). More so, *Ficus glumosa* showed same inhibition zone of 20.33±0.58 mm against *Streptococcus pyogenes* while least activity of 7.67±0.58was recorded against *E. coli.* The ethanolic leaf extracts of both extracts revealed the presence of cardenolides, tannins, flavonoid, saponins and cardiac glycoside. Terpenoids was only detected in *F. glumosa*. However, both extracts were devoid of alkaloids and anthraquinones. Therefore, the high activity posed by *C. capitatum* against most organisms studied compared to *F. glumosa* may possibly be due to high concentrations of these secondary metabolites in the former and hence suggestive for its choice in the treatment of the aforementioned microorganisms.

Keywords: Antimicrobial, *Clerodendrum*, Comparative, *Ficus*, Leaf, Phytochemistry

1. Introduction
The use of medicinal plants to treat various ailments is as old as the world (Bennett and Brown, 2000) [2]. It is well known throughout history and has always been part of human culture. The World Health Organization (WHO) estimates that up to 80% of the world's population relies on traditional medicinal system for some aspects of primary health care. In African ethno medicines (Bennett and Brown, 2000) [2], it is well known that traditional healers make use of a large variety of herbs in the treatment of parasitic diseases including malaria and a wide proportion of herbal remedies dispensed by traditional healers are widely believed by their clients to be effective. Such plants can play an important role in drug discovery and their studies are logical research strategies in search for new drugs (Kirby, 1997) [8]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Gislene *et al.*, 2000) [6]. Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality. From 1980 to 1990, Montelli and Levy (Gislene *et al.*, 2000) [6] documented a high incidence of resistant microorganisms in clinical microbiology all over the world. According to World Health Organization (Gislene *et al.*, 2000) [6] medicinal plants would be the best source to obtain a variety of drugs with over 80% of the populace from developing countries use traditional medicine.

Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Gislene *et al*., 2000) [6]. In this study, ethanolic extracts of two different plant belonging to different family used in traditional medicine either singly or in combinations in some parts of Northern Nigeria were screened for their antimicrobial potency.

Clerodendrum capitatum (Wild.) Schum. & Thonn. belongs to the Verbenaceae family, locally named "*Bambaro* or *Maashayi* also as *Taabaataabaa*" in Hausa and "*Korlejiga*" in Fulfulde (Burkill, 2000) [4]. *Clerodendrum* is a very large and diverse genus with about 580 identified species. It is the largest genus of the tribe Teucrieae (Steane *et al*., 1999) [15]. It is widely distributed in the tropical and warm temperate regions of the world, with most of the species occurring in tropical Africa and Asia (Rueda, 1993) [11]. It possesses potent anti-inflammatory, anti-intecctive and anti-oxidant activities among others (Adeneye *et al*., 2008) [1].

Ficus glumosa, commonly known as fig tree or "Africa rock fig"in English, Hausa; Kawuri Soomaaliga: Berde. is a plant with immense medicinal value, the sacred trees of religious values in ancient times mentioned in religious scriptures such as the Qur'an and the Bible. It is a large tree belonging to the family moraceae comprising of other species, including *Ficus religiosa* and *Ficus benghanlensis*. *Ficus glumosa* is a shrub with creeping stem found in rocky beds of fast-flowing streams that are exposed to two alternate environments. *Ficus glumosa* is indigenous to the tropical and subtropical Africa including Nigeria (Sastrava *et al.*, 2003). The latex is applied to alleviate pain from sprains and when diluted in water is used to treat diarrhea, it is also used as a dropper in the eyes against sore eyes. In East Africa pounded bark are soaked in water to drink against stomach disorders. In Nigeria leaf are used against blood glucose levels and in diabetic rats (Sastrava *et al.*, 2003).

Materials and Methods

Sample collection and identification

The two samples *Clerondendrum capitatum* and *Ficus glumosa* were separately collected from Demsa Local Government Area, and Nkafimiya village, Michika Local Government Area, Adamawa State, Nigeria. Plant specimens were identified and authenticated by a plant Taxonomist from the Department of Biological Sciences, University of Maiduguri. The collected plant were separately handpicked and air dried for 3-5 days, freed from twigs and dirt, they were crushed to coarse powder using mortar and pestle, labeled and then stored in a plastic bag until extraction.

Extraction of plant materials

The air-dried powered plant materials of *C. capitatum* and *F. glumosa* (250 g) each were extracted exhaustively with 70% methanol in distilled water using soxhlet apparatus as described by Lin *et.al.* (1999) [9]. The combined methanolic extracts were concentrated to dryness at reduced pressure using rotary evaporator and there were coded as "CCME"- *C. capitatum* and "FGME"- *F. glumosa* methanol extract. They were then subjected to qualitative phytochemical screening and *in vitro* antimicrobial susceptibility test.

Phytochemical screening

Qualitative analysis of the crude extracts was carried out as described by Harborne (1973) [7], Brain and Turner (1975), Vishnoi (1979) [20], Markham (1982) [10], Farnsworth (1989) [5], Sofowora (1993) [14], Silver *et al.* (1998) [13] and Trease and Evans (2002) [16], to identify the presence of the classes of compounds such as alkaloids, anthraquinones, carbohydrate, flavonoids, tannins, saponins, glycosides, cardiac glycosides, steroidal glycosides and terpenes/terpenoids.

Antimicrobial studies

Test microorganisms

The Gram positive organisms used in this study were *Bacillus subtilis, Staphylococcus aureus,* and *Streptococcus pyogenes* while Gram negative organisms were *Escherichia coli,, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and one fungal species *Candida albicans.* These organisms were purified clinical laboratory isolates obtained from the Department of Veterinary Medicine, University of Maiduguri, Maiduguri, Nigeria.

Disc diffusion technique

The method of Vollekovà *et al*. (2001) [21] as modified by Usman *et al*. (2005) [17] was adopted. The two samples: *C. capitatum* and *F. glumosa* were tested on the 7 isolates at different concentrations. Three holes were bored in each plates (6 mm diameter) using sterile cork-borer. About 0.2 ml of the extract was inoculated across the wells and inoculated at 37 ^{oC} for 18-24 hrs. After incubation, the average diameter of three readings of the clear zone surrounding the hole was taken as the measure of the inhibitory level of plant extract against the bacteria on test and recorded as mean ± SEM.

Results

The results of these analyses are presented in the Tables below. Table 1 presents the phytoconstituents of the two extractives. In Table 2 are found the susceptibility pattern of the two plant extracts at different dosages which are reported as zones of inhibition diameters. As a result of the activities of the extractive, the minimum inhibitory and minimum bactericidal concentrations were estimated as shown on Tables 3 and 4 below.

Table 1: Phytochemical evaluation of *Clerodendrum capitatum* and *Ficus glumosa* leaf extracts

Phytoconstituents	Test	Extract Type	
		Clerodendrum capitatum	***Ficus glumosa***
Alkaloids		-	-
Anthraquinones			
Free	Bontrager's	-	-
Combined	Bontrager's	-	-
Saponins	Frothing	+	+
Carbohydrates		+	+
Cardenolides			
	Keller-Kiliani's	+	+
Cardiac glycoside			
Steroidal nucleus	Salkowski's	+	+
Terpenoids	Liebermann-Burchard's	-	+
Flavonoids	Shinoda's	+	+
Tannins	Ferric chloride test	+	+
Phlobatannins		-	-

Key: + = positive; - = negative

Table 2: Antimicrobial susceptibility of the leaf extract for *Clerodendrum capitatum* and *Ficus glumosa* at various concentrations

Microorganisms	Plant extract	Concentration of the extract/Diameter inhibition zone (Mean±SEM)		
		80 mg/hole	40 mg/hole	20 mg/hole
Bacillus subtilis	*C. capitatum*	0.00±0.00	0.00±0.00	0.00±0.00
	F. glumosa	11.66±0.58	9.00±0.00	0.00±0.00
Staphylococcus aureus	*C. capitatum*	14.33±0.58	10.33±0.58	7.33±0.58
	F. glumosa	0.00±0.00	0.00±0.00	0.00±0.00
Streptococcus pyogenes	*C. capitatum*	14.33±0.58	10.00±0.00	7.00±0.00
	F. glumosa	20.33±0.58	17.00±0.00	13.00±0.00
Escherichia coli	*C. capitatum*	14.67±0.58	10.67±0.58	7.67±0.58
	F. glumosa	10.00±0.00	7.00±0.00	0.00±0.00
Klebsiella pneumoniae	*C. capitatum*	15.67±0.58	11.67±0.58	8.33±0.58
	F. glumosa	0.00±0.00	0.00±0.00	0.00±0.00
Pseudomonas aeruginosa	*C. capitatum*	12.00 ±0.00	9.00±0.00	0.00±0.00
	F. glumosa	0.00±0.00	0.00±0.00	0.00±0.00
Candida albicans	*C. capitatum*	20.33±0.58	16.33±0.58	12.33±0.55
	F. glumosa	16.67±0.58	12.66±0.58	9.00±0.00

Discussions

The preliminary phytochemical screening of the three ethanolic extracts (is shown in Table 1. This reveals the presence of cardiac glycosides, flavonoids, terpenes, steroids and tannins in most of the plant extracts studied. Terpenoids was absent in CCME, while alkaloids were not detected in all the extracts. These secondary plant metabolites have been reported by many authors to be responsible for most pharmacological and biological affects both *in vitro* and *in vivo* exhibited by plants extracts. All the extracts showed a considerable amount of tannin and flavonoids, tannins have being reported. Tannins have been reported to inhibit the growth of microorganism by precipitating microbial protein and making nutritional protein unavailable to them (Usman *et al.,* 2009) [19] the inhibitory activities of gallotannins are attributable to their strong affinity for iron and likely additionally relate to the inactivation of membrane-bound proteins (Usman and Osuji, 2007 [18]. Flavonoids exhibit s antimicrobial property through their ability to complex with extracellular, soluble protein and to complex with bacterial cell wall proteins (Usman *et al.,* 2009) [19]. Saponins have been reported to possess a wide range of biological activities especially antibacterial whose mode of action involved cell membrane lysis (Usman *et al.,* 2009) [19].

The results of the inhibition zone diameters of the two extracts were presented in Table 2. The diameter of inhibition zones exhibited by CCME had the ranges of 14.33±0.58 to 0.00, 15.67±0.58 to 0.00 and 20.33±0.58 to 0.00 mm values for Gram positive, Gram negative and fungal respectively and FGME value for Gram positive, Gram negative and fungal ranges from 20.33±0.58 to 13.00±0.00 mm, 10.00±0.00 to 0.00 mm and 16.66±0.58 to 9.00±0.00 mm respectively. The overall susceptibility data revealed that *Clerodendrum capitatum* was highly effective on *Candida albicans* while it was least active on *Staphylococcus aureus.* More so, *Ficus glumosa* showed same inhibition zone value of 20.33±0.58 mm against *Streptococcus pyogenes* while least activity was recorded against *E. coli.* The fact that both extracts contained almost the same composition of secondary plant metabolites that does not relate to their wholly therapeutic activities, due possibly to several factors such as the classes of the compounds, masking and synergistic effects among others numerous to mention. From these findings, it is therefore, pertinent to say that the extracts could be a good source of cure against superficial and deep follicular wound infections particularly those whose causative agents are among those organisms that showed high activities.

Conclusion

This study has shown that the ethanolic extract of *Clerodendrum capitatum* shows good antifungal activity of *Candida albicans* at a relative concentration possibly against candidiasis while *F. glumosa* may be good candidate against *Streptococci* related infections. Therefore, the use of these plants should be with caution to avoid misconception. The overall assessment confirms that *Clerodendrum capitatum* is comparatively more effective than *Ficus glumosa* on most the organisms under study.

Acknowledgements

The authors wish to acknowledge the technical efforts of Mr. Fine Akawo of Chemistry Department and Alhaji Isa Adamu Gulani of Department of Veterinary Medicine, University of Maiduguri.

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