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Phytochemical, IR Spectral and Biological Studies on the Leaf Extracts of *Commiphora Africana* (Burseraceae)

Isyaka Mohammed Sani and Okwute Simon Koma
Department of Chemistry, University of Abuja, P. M. B. 117, Gwagwalada, Abuja, F. C. T., Nigeria.
Email: profokwute@yahoo.com

ABSTRACT

The dry powdered leaf of *Commiphora africana* was extracted with methanol to give the crude extract. The crude extract was subjected to phytochemical analysis which revealed the presence of tannins, flavonoids, triterpenoids, saponins, and alkaloids among other classes of natural products. The crude extract was fractionated into n-hexane, diethyl ether, and n-butanol soluble fractions. The crude extract was active against *Baciillus subtillis* at MIC value of 1000µg, and *Staphylococcus aureus* and *Escherichia coli* at 2000µg, while the n-butanol was active only against *Bacillus subtillis* at 1000µg. However, the n-hexane and diethyl ether fractions were not active against any of the test organisms even at 2000µg. The IR spectra of crude extract and fractions showed the presence of hydroxyl, carbonyl and aromatic systems which are in good agreement with results of the phytochemical analysis of the crude extract.

Keywords: Antimicrobial, *Commiphora africana* , extracts, IR, phytochemical.

INTRODUCTION

*Commiphora Africana (*Burseraceae) is widely used in the northern parts of Nigeria as an incensing, insecticidal and antiseptic fumigant(Dalziel, 1937). Previous chemical investigations of the species of the genus, *Commiphora*, have been on the resins which yielded various classes of terpenoids and a lignan (Thomas and Willhalm, 1964; Carl and Noble, 1980; Carl and Noble, 1983; Waterman and Amopofo, 1985; Provan and Waterman, 1985; Provan and Waterman, 1986). Most of these earlier investigations did not screen any extractives for biological activities. However, in one of the investigations some extracts of the root of *C. africana* were found to possess antimicrobial activity while β-sitostenone and α-amyrin were isolated from the n-hexane fraction (Okwute, 1989). More recent investigations included the study on the anti-inflammatory and analgesic effects of the hydro-ethanolic extract of Commiphora Africana stem bark on rodents (Ezekiel, 2010), and that on the leaf, employing hydro-distillation and GC-MS analysis, which focused on the anti-oxidant activity of the essential oil of the species from the West African country, Benin(Ayeodoun *et. al.*, 1998; Choudhury *et. al.*, 2000; Ma *et. al*., 2004, Avlessi et. al., 2005). The leaf was found to contain mostly sesquiterpenoids with the bisbolane skeleton, dimethyl-terephthalate, and a dihydroflavanol, but no reasonable antiradical property was observed.

In our continued study of the species *Commiphora africana* as a source of anti-infective and anti-inflammatory agents we decided to perform photochemical and antimicrobial screening of the crude extract and fractions of the leaf as a prelude to further work on the search for active constituents of the plant. The preliminary results are reported in this paper.

MATERIALS AND METHODS

The leaf of *Commiphora africana* (*dashi*=Hausa) was collected from Gumau, Toro Local Government Area of Bauchi State in May, 2008, by Mall. Abubakar Nukrah. It was authenticated at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria. It was air-dried and powdered.

All reagents used in this work were of standard grade and the solvents were re-distilled before use. TLC was run on pre-coated aluminum sheets and spots were detected using both iodine vapour and UV lamp (366+254 nm). IR measurements were obtained on Genesis Series ATI Mattson and values are recorded in wave numbers (cm^{-1}).

The crude extract and fractions were dissolved in DMSO for the antimicrobial screening. The Agar used for the antimicrobial screening was the Mueller Hiutan. The organisms for the antimicrobial screening included *Bacillus subtillis Staphylococcus aureus, Escherichia coli,*

Salmonella typhi, Plebsiella spp., and *Candida albicans* and were available at NIPRD. Incubations after inoculations were done at 37^{o}C for 24 hours.

The powdered leaf (20g) was extracted exhaustively with methanol using a Soxhlet Extractor. After filtration by suction, the solution was evaporated to dryness using a Rotavapor to give a greenish black residue (32% of dry plant material).

The crude extract was also analysed for phytochemicals according to standard procedures (Harbone, 1973). The crude methanol extract was dissolved in 400 ml of 50% aqueous methanol and fractionated into n-hexane (1.92g), diethyl ether (2.21g), and n-butanol (2.60g) soluble fractions in a separating funnel. The crude extract and fractions were also subjected to IR spectral analysis. The crude methanol extract and fractions were subjected to antimicrobial screening against some human pathogens using agar-streak dilution technique (Mitscher *et. al.,* 1972).

RESULTS AND DISCUSSION

The results of phytochemical screening of the crude methanol extract of the leaf of *Commiphora africana* are shown in Table 1. The IR spectrum of one of the fractions, the hexane-soluble, is also presented in Figure 1**.**

Table 1: Phytochemical screening results of crude methanol extract of leaf of Commiphora africana.

Phytochemicals	Remark
Tannins	+
Flavonoids	+
Anthraquinones	+
Glycosides	-
Triterpenoids	+
Saponins	+
Balsams	-
Sterols	+
Phenols	+
Reducing sugars	+
Alkaloids	+
Cardohydrates	-
Phlobatannins	+

Key: (+)= present; (-)=absent

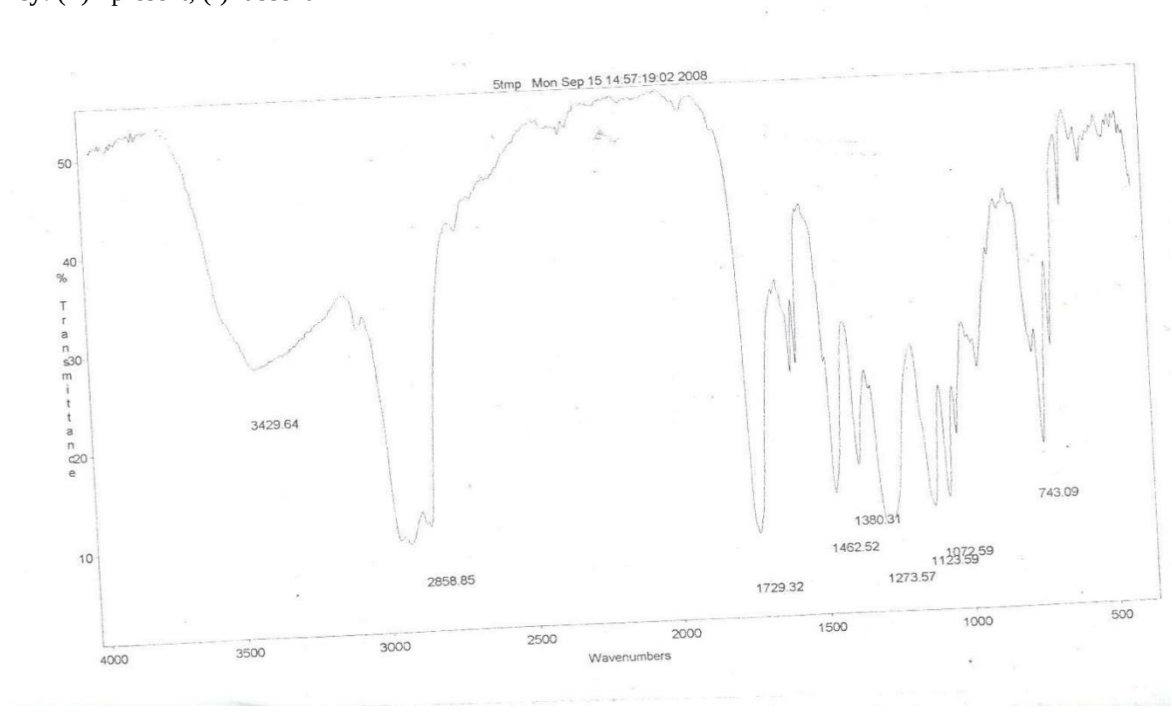

Figure 1: IR spectrum of n- hexane fraction of methanol extract of leaf of *Commiphora africana*

The results of phytochemical analysis (Table 1) showed the presence of a wide range of classes of natural products which include terpenoids, triterpenoids, phenols, flavonoids and alkaloids, but no glycosides and complex carbohydrates. This is in good agreement with previous reports on other species of *Commiphora* (Waterman and Ampofo, 1985; Provan and Wateman, 1985; Banso and Mann, 2006) and this is partly supported by the IR spectral characteristics of extractives from the leaf as typified by IR spectrum of the hexane fraction (Figure **1**). Thus, the OH, C-H, C=O, Aryl C=C, and C-O absorptions at about 3429.64, 2858.85, 1729.32, 1605, and 1462.52 cm^{-1} are indicative of the presence of phenols, flavonoids, and triterpenoids in the leaf extracts. The dry weight of most species of *Commiphora* is usually very light and may be suggestive of very low carbohydrate content. While no glycoside was detected in this work a glucoside was isolated by previous workers from the leaf of *Commiphora africana* from Benin (Ma *et. a*l., 2004). Also, while the presence of antimicrobial alkaloids has been reported previously only in one case and from the stem bark of *Commiphora Africana* (Banso and Mann, 2006), this work has detected alkaloids in the leaf extract and this may be an exciting observation.

The antimicrobial screening results (Table 2) showed that essentially only the crude methanol extract and the n-butanol fraction were active.

Table 2: Antimicrobial screening results of extractives from leaf of Commiphora africana

Extractives	Organisms(MIC, µg/ml)					
	Bs	Sa	Ca	Ps	St	Ec
Methanol 2mg 1mg 0.5mg	+ + -	+ -	-	-	-	+ +*
n-Hexane 2mg	-	-	-	-	-	-
Diethyl ether 2mg	-	-	-	-	-	-
n-Butanol 2mg 1mg 0.5mg	+ + -	-	-	-	-	-

Key: (+)= active; (+)*=weakly active; (-)=inactive
Bs=*Bacillus subtillis;* Sa=*Staphylococcus aureus;* Ca=*Candida albicans*;
Ps=*Plebsiella spp*; St=*Salmonella typhi*; Ec= *Escherichia coli*.

While the crude methanol extract was active against both *Staphylococcus aureus* and *Escherichia coli* at MIC value of 1000µg, the n-butanol fraction was active only against *Staphylococcus aureus* and at MIC of 1000µg, among the test organisms. Thus, both the n-hexane and diethyl ether fractions were not active against the test organisms, including *Staphylococcus aureus* which the n-hexane and the diethylether fractions of the root extract were previously reported to be active against and at MIC of 100µg (0kwute 1989). Therefore, between the root and leaf of *Commiphora africana*, the former is more potent as an antimicrobial agent. However, the potency at 1000µg is good enough to excite further work on the polar n-butanol fraction. This will be the subject of a future report on this important medicinal plant which is widespread in the Sahel region of Africa.

CONCLUSION

The results obtained of the phytochemical fanraomlysis showed that the leaf extracts of *Commiphora africana* contains a wide range of phytochemicals and exhibits broad spectrum pharmacological activities. It contains tannins, flavonoids, triterpenoids, saponins, anthraquinones, sterols, phenols, reducing sugars, phlobatanins and alkaloids which are generally known to have protective effects against several diseases. Specifically, the work has confirmed that the leaf of *commiphora africana* possesses a broad spectrum antimicrobial activity against the test organisms leading to the conclusion that *commiphora africana* has very significant biological activities that are of relevance in human healthcare.

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