



## PHYTOCHEMICAL DETERMINATIONS AND ANTIBACTERIAL ACTIVITIES OF THE LEAF EXTRACTS OF *COMBRETUM MOLLE* AND *GOSSYPIUM ARBOREUM*

Saidu, T.B. and Abdullahi, M.

Department of Basic and Applied Sciences Niger State Polytechnic, Zungeru, Niger State

\*Correspondence author: talatusaidu@yahoo.com

### ABSTRACT

An investigation was carried out to screen the leaf extracts of *Combretum mole* and *Gossypium arboreum* for the presence of phytochemical components and antibacterial activity against *Staphylococcus aureus* strain OE5, *Pseudomonas aeruginosa* strain OE9, *Klebsiella pneumoniae* strain OE8 and *Escherichia coli* strain OE7 isolated from cases of otitis externa, using the agar diffusion procedure. The results indicated that the two medicinal plants contained alkaloids, tannins, saponins, anthracene, phenols and sesquiterpenes. Volatile oil was detected only in *G. arboreum*. The aqueous, ethanol and methanol extracts of the plants inhibited the growth of all the test organisms to varying degrees with the exception of *E. coli*, which was resistant to aqueous extract of *C. mole*. However, overall, the extracts of *G. arboreum* showed a higher degree of inhibition of the test bacteria than *C. mole*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of *G. arboreum* and *C. mole* varied and ranged from 0.31mg/ml to 2.5mg/ml and 2.5mg/ml to 5.0mg/ml respectively. Synergistic effect of the ethanol and methanol extracts of the two plants resulted in enhanced antibacterial activity (large zones of inhibition, lower MIC, 0.31 – 1.25mg/ml, MBC, 0.63-2.5mg/ml).

**Keywords:** Extracts, inhibition, plants, pathogens, screening.

### INTRODUCTION

The development of antibiotics for treatment of diseases is being intensified which results in the development of new ones but bacteria are busy evolving resistance to the antibiotics and proliferate in the environment to cause harm. Antimicrobial activity of medicinal plants have been reported by other researchers (Martinez *et al.*, 1996; Manna and Abalaka, 2000; Fyhrquist *et al.*, 2002; Onyeagba *et al.*, 2004; Babayi ., 2004, Manna *et al.*, 1997) reported strong activity of leaf extracts of *Calotropis procera* against *Clostridium perfringens*, *Streptococcus faecalis*, *Klebsiella ozaenae*, *Pseudomonas aeruginosa* and *Salmonella typhi*. On the activity of specific phytochemical components, Takeda and Fatope (1998) isolated lawsoniaside and laliside from the ethanol extracts of the leaves of *Lawsonia inermis*, which has medicinal value in Northern Nigeria, the Middle East and Asia. Saxena (1990) reported an isolate of the neem plant, azadirachtin, with pharmaceutical properties, confirming that, the plant has medicinal value. Saxena (1990) reported the effects of alkaloids and cardiac glycosides obtained from *Garcinia kola*, *Borreria ocyroides*, *Kola nitida* and *Citrus aurantifolia* on pathogenic bacteria. Similarly, Ijah and Oyebanji (2003) reported that tannins and polyphenols obtained from *Enantia chlorantha*, *Kigelia africana*, *Bridelia ferruginia*, *Trema nitens* and *Drypetes gossweileri* inhibited the growth *Staphylococcus aureus*, *Klebsiella* spp, *Proteus* spp, and *E. coli* but had no inhibitory effect on *Pseudomonas aeruginosa*.

The present study was undertaken to screen *Combretum mole* and *Gossypium arboreum* for

phytochemical components and to test the leaf extracts of the plants for activity on some bacterial pathogens of otitis externa

### MATERIALS AND METHODS

#### Sources, identification, and processing of plant materials

The leaves of two medicinal plants used locally for the treatment of otitis externa were collected from Gurusu village near Minna, Niger State, Nigeria. The plants were identified as *Gossypium arboreum* and *Combretum mole* (Irobi and Daramola, 1993). The leaves of the plants were air dried to keep photocells intact for three days, pounded in a mortar with pestle and stored in sterile bottles for further study.

#### Test bacteria used

Bacteria used in this study were *Staphylococcus aureus* strain OE5, *Escherichia coli* strain OE7, *Klebsiella pneumoniae* strain OE8 and *Pseudomonas aeruginosa* strain OE9. These microorganisms were isolated from cases of otitis externa in patients who attended the General Hospital, Minna, Nigeria (Ijah *et al.*; 2003)

#### Extraction Procedures

Twenty five grammes (25g) of the leaf powder of each plant was soaked in 100 ml of distilled water, 95% ethanol and methanol (BDH chemical, Poole, UK) in Erlenmeyer flasks and allowed to soak for 72hours (Irobi and Daramola, 1993). The percolate obtained was passed through a Whatman filter paper No.1 (Whatman, UK) and concentrated in vacuo using a rotary evaporator (Buchii laboratory Technique, Switzerland) at 40 degree centigrade.

The extracts were stored in sterile screw capped bottles and kept in the refrigerator (Thermocool Engineering Company Limited, Ikeja, Nigeria) at 4°C for further study.

#### **Phytochemical screening of crude extracts of *Combratum mole* and *Gossypium arboreum***

The phytochemical components of the medicinal plants were tested using the method of Harbone (1984) and Trease and Evans (1989). The various tests are described below:

- i) Test for anthracenes  
One milliliter [1.0ml] of the plant extract was shaken with equal volume of chloroform and 10% ammonia solution was added to the chloroform layer. Formation of red precipitate indicated the presence of anthracene.
- ii) Test for tannin  
Four milliliter (4.0 ml) of water was added to 0.5ml of the plant extract; drops of ferric acid chloride were then added. The immediate development of green precipitate indicated the presence of tannin. The presence of tannin was further confirmed by the development of red colouration on addition of lead acetate to the extract.
- iii) Test for volatile oils  
One milliliter (1.0ml) of the plant extract and 0.1ml of dilute sodium hydroxide [NaOH] solution and dilute hydrochloric acid [HCl] were mixed. The formation of a white precipitate indicated the presence of volatile oils.
- iv) Test for saponins  
Five gramme (5g) of the crude extract was added to 10ml of water and shaken for 2 minutes. Frothing which persisted on warming was an evidence of the presence of saponins.
- v) Test for alkaloids  
Five drops of 100% tannic acid was added to 0.5ml of the extract. Formation of cream colour indicated the presence of alkaloids. Confirmatory test for alkaloids was done by adding drops of Dragendorff's reagent to the extract and the development of deep brown precipitate confirmed the presence of alkaloids.
- vi) Test for sesquiterpenes  
Aqueous extract (0.5 ml) of the plant was mixed with 0.1ml of methanol and shaken vigorously. To this mixture, 0.4ml of 5% sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) plus 0.5% ferric chloride was added. The content was stirred using a small glass rod. The mixture was boiled in a water bath for one minute. A change in colour from colorless to pink indicated the presence of sesquiterpenes.
- vii) Test for phenols  
Equal volumes (0.2ml) of the plant extracts and ferric chloride (FeCl<sub>3</sub>) were mixed. The development of deep bluish green colour indicated the presence of phenols.

#### **Determination of Antibacterial activity of plants extracts**

Well diffusion method of Collins *et al.* (1995) was used in this test. About 0.2g of each plant extract was

dissolved in 5ml of distilled water, ethanol and methanol respectively. Three hour peptone water grown culture of the test organism was inoculated on nutrient agar plates. Holes of 6mm diameter were created in nutrient plates using a cork borer and 0.2ml of the extracts was added to each hole. The plates were incubated at 30°C for 24 hours.

The development of zones of inhibition around the holes containing the extracts indicated the antimicrobial activity of the plant extracts against the test microorganisms.

#### **Determination of Minimum Inhibitory Concentration (MIC) and Minimum bactericidal concentration (MBC) of plant extracts**

About (0.5mls) of the reconstituted extracts (20mg/ml) of *C. mole* and *G. arboreum* was placed in test tubes (number 1- 8) containing peptone water. The first tube contained 1.5ml of peptone water while tubes 2-8 contained 1.0ml of peptone water each. Tube 8 served as the control experiment. After serial dilution, the final concentration of each tube was 10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml, 0.63mg/ml, 0.31mg/ml and 0.155mg/ml respectively. The tubes were inoculated with a loopful of the test microorganisms and incubated at 37°C for 24h. At the end of the incubation, the lowest concentration which showed no visible turbidity (growth of microorganism) was regarded as the minimum inhibitory concentration (Prescott *et al.*, 1990). The same procedure (as described above) was used for determination of MIC of the combined extracts of *C. mole* and *G. arboreum* and for alkaloids and tannins. The test solutions for the MIC were plated out to determine the MBC of the extracts (Babayi *et al.*, 2004).

#### **RESULTS**

Phytochemical analysis of the extracts of *C. mole* and *G. arboreum* showed that anthraquinone and cardiac glycosides were not present in both plants extract (Table 1). Volatile oils were detected in *G. arboreum* only while alkaloids, tannins, phenols, sesquiterpenes, anthracene and saponins were present in both plants. The results (Table 2) showed that the extracts of the two medicinal plants exhibit varying antimicrobial effects on *S. aureus* strain OE5, *K. pneumoniae* strain OE8, *E. coli* strain OE7, and *P. aeruginosa* strain OE9. *Combratum mole* showed a higher activity on the bacteria than *G. arboreum* (Table 2). A combination of the extracts of the two plants produced larger zones of inhibition than a single plant extract used (Table 2). The MIC of the extract ranged from 1.25mg/ml to 2.5 mg/ml for *C. mole* and 0.63mg/ml to 1.25mg/ml for *G. arboreum* extracts (Table 3). Similarly the MIC of the combined plant extracts was 0.31mg/ml – 1.25mg/ml. The MBC for extracts of *C. mole* ranged from 2.5mg/ml to 5.0mg/ml while that of *G. arboreum* was 1.25mg/ml to 5.0mg/ml. The combined extracts of the medicinal plants had a MBC of 0.63mg/ml to 2.5mg/ml, which is much lower than the MBC for individual plant extracts (Table 4).

**Table1: Phytochemical components of leaf extracts of *Combratum mole* and *Gossypium arboreum***

Phytochemical Components	Combratum mole			Gossypium arboreum		
	EE	ME	AE	EE	ME	AE
Alkaloids	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-
Anthracene	+	+	+	+	+	+
Cardiac glycosides	-	-	-	-	-	-
Phenol	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Sesquiterpenes	+	+	+	+	+	+
Tannin	+	+	+	+	+	+
Volatile oils	-	-	-	+	+	+

Key: + present, - : Absent. EE : Ethanol extract, ME : Methanol extract. AE: Aqueous extract

**Table 2: Sensitivity (mm) of Bacteria to leaf extracts of *Combratum mole* and *Gossypium arboreum* using agar diffusion method**

Bacteria	<i>Combratum mole</i>			<i>Gossypium arboreum</i>			<i>C. mole</i> Plus <i>G. arboreum</i>		
	EE	ME	AE	EE	ME	AE	EE	ME	AE
<i>S. aureus</i> strain OE5	20.0± 0.5	18.2±0.8	10.0±0.2	10.0±0.0	8.0±0.3	10.0 ±0.2	31.2±1.2	29.0±0.6	25.0±0.8
<i>E. coli</i> strain OE7	10.0±0.0	10.0±0.2	0.0	10.0±0.0	8.4±0.3	8.0±0.4	30.1±0.7	30.0±1.4	25.0±0.4
<i>K. pneumoniae</i> Strain OE8	20.0±1.2	20.0±0.6	15.0±0.4	15.0±0.6	15.0±0.6	12.0±0.5	29.4±1.2	30.0±1.3	28.0±0.6
<i>Ps. aeruginosa</i> Strain OE9	20.0±0.4	10.0±0.0	10.0±0.4	18.0±0.0	15.0±0.4	10.0±0.6	30.0±0.8	28.0±0.8	24.0±0.7

EE: Ethanol extract, ME Methanol extract, AE: Aqueous extract; a: standard deviation is based on two determinations

**Table 3: Minimum inhibitory concentration (MIC) of extracts of *C. mole* and *G. arboreum* extracts**  
Minimum inhibitory concentration (mg/ml) of extracts

Bacteria	<i>C. mole</i>			<i>G. arboreum</i>			<i>C.mole</i> Plus <i>G.arboreum</i>		
	EE	ME	AE	EE	ME	AE	EE	ME	AE
<i>S. aureus</i> strain OE5	1.25	1.25	1.25	1.25	1.25	1.25	0.31	0.31	1.25
<i>E. coli</i> strain OE7	2.5	2.5	2.5	0.63	0.63	1.25	0.31	0.31	1.25
<i>K. pneumoniae</i> Strain OE8	2.5	2.5	2.5	0.63	0.63	1.25	0.31	0.31	1.25
<i>Ps. aeruginosa</i> Strain OE9	1.25	1.25	1.25	1.25	1.25	1.25	0.31	0.31	1.25

Key: EE: Ethanol extract, ME: Methanol extract, AE: Aqueous extract

**Table4: Minimum bactericidal concentration (MBC) of extracts of *C.mole* and *G. arboreum* Extracts**  
Minimum bactericidal concentration (mg/ml) of extracts

Bacteria	<i>C. mole</i>			<i>G. arboreum</i>			<i>C.mole</i> Plus <i>G.arboreum</i>		
	EE	ME	AE	EE	ME	AE	EE	ME	AE
<i>S. aureus</i> strain OE5	2.5	2.5	5.0	1.25	1.25	2.5	0.63	0.63	2.5
<i>E.coli</i> strain OE7	5.0	5.0	5.0	2.5	2.5	5.0	0.63	0.63	0.63
<i>K.pneumoniae</i> Strain OE9	5.0	5.0	5.0	2.5	2.5	5.0	0.63	0.63	0.63
<i>Ps. aeruginosa</i> Strain OE9	5.0	5.0	5.0	2.5	2.5	2.5	0.63	0.63	0.63

Key: EE: Ethanol extract, ME: Methanol extract, AE: Aqueous extract.

## DISCUSSION

The ethanol, methanol and aqueous extracts of *C.mole* and *G. arboreum* analysed contained one or more of the following phytochemical components; alkaloids, volatile oil, tannins, phenols, saponins,

sesquiterpenes and anthracene. Similar active compounds have been reported in other medicinal plants including members of the family Combrataceae (Brantner and Grein, 1994; Ijah and Sar, 1998; Ijah and Oyebanji, 2003; Babayi *et al.*, 2001).

The inhibitory effects of the medicinal plants on the test bacteria may therefore, be due to the presence of these phytochemical components, which have been reported to be responsible for the antibacterial activities of certain medicinal plants (Martinez *et al.*, 1996; Ijah and Oyebanji, 2003; Omojasola and Awe, 2004). The results of the Bioassay study showed that the combined extracts of the two plants caused greater antibacterial activity (yielded much larger zones of inhibition). This imply that extracts of the plants can be more effective when used in combined form to treat diseases (Gill, 1992; Bone, 1994; Babayi *et al.*, 2004) reported an enhanced effectiveness of the combined fractions of the extracts of *Eucalyptus camaldulensis* and *Terminalia catapa* on the growth of *C. albicans*, *S. aureus*, *E.coli* and *B.subtilis*

The MIC of the extracts of *C.mole* ranged from 1.25mg/ml to 2.5mg/ml while that *G. arboreum* ranged from 0.31mg/ml to 1.2mg/ml. This means that higher concentrations of the extracts from *C.mole* is required to inhibit the growth of the test organisms where as low concentration of extracts of *G. arboreum* is required to inhibit the growth of the organisms particularly *E.coli* strain OE7 and *K. pneumoniae* strain OE8. Thus extracts from *G. arboreum* proved very effective on the test organisms, even at lower concentrations. The minimum bactericidal concentration (MBC) was found to be higher for individual plant extracts than the combined extracts of the two plants, meaning that a higher concentration of the individual extracts is required to exert cidal effects on the bacteria. The considerably low MIC and MBC of the combined extracts may be attributed to the synergistic activities between the phytochemical components. This further stresses the importance of using the plant extracts in a combined form to achieve a more effective treatment of diseases.

## REFERENCES

- Babayi, H., Kolo, I., Olokun, J.I and Ijah, U.J.J (2004). The antimicrobial activities of methanoic extracts of *Eucalyptus camaldulensis* and *Terminalia catappa* against pathogenic microorganisms. *Biokemistri* 16(2): 106-111.
- Benjamin, T.V. (1998). Investigation of *Borrielia vericuplata*. *Quarterly journal of Crude Drug Research* 17(2): 72 – 80
- Bone, K. (1994). Dosage considerations in herbal medicine. *British Journal of Phytotherapy* 3(3): 128 – 137.
- Branter, A. and Grein, E. (1994). Antibacterial activity of plants extracts used externally in traditional medicine. *Journal of Ethnopharmacology* 44: 35 – 40
- Collins, C.H., Lynes, P.M and Grange, J.M (1995). Microbiological exploitation of

Benjamin (1989) reported that phytochemical components of the medicinal plants affect bacteria in different ways. In the present study, the plant extract exerted antimicrobial effects on all test organisms with the exception of *P. aeruginosa* strain OE9.

## CONCLUSION

This study has established the scientific basis for the traditional use of *C. mole* and *G. arboretum* in the treatment of otitis externa. The combined extracts of the two plants were found to be effective on the test organisms than single plant extracts. The ethanol and methanol extracts of the plants were more active in inhibiting growth of the organisms than its crude aqueous extracts of the plant, meaning that the active ingredient were more soluble in alcohol than in water. The results suggest that the two plants, *C. mole* and *G. arboreum* can be employed in the treatment of otitis externa.

## RECOMMENDATIONS

Based on the results obtained from this study the following recommendations are made:

1. These plants resources should be conserved under in situ environment because of their medicinal values.
2. Toxicity studies on the active ingredient together with clinical trial to determine their potential use as chemotherapeutic agents should be undertaken.
3. More research work should be encouraged to further determine the efficacy of extracts of *C. mole* and *G. arboreum* for the treatment of otitis media.
4. The chemical structure of the active ingredients present in the plant extracts should be determined.

cardiacglycosides and alkaloids from *Garcina kola*, *Borreria ocymoides*, *Kola nitida* and *Citrus aurantifolia*. *Journal of Applied Bacteriology* 71: 398 – 401

- Fyhrguist, P., Nwassumbi, L., Haeggstrom, C., Vuorela, H., Hiltinen, R. and Vuorela, P. (2002). Ethnobotanical and antimicrobial investigation on some species of *Terminalia* and *Combratum* (Combretaceae) growing in Tanzania. *Journal of Ethnopharmacology* 79: 169 – 177.
- Funatogawa, J., Hayashi, S., Shimomura, H., Yoshida, T., Hakano, T., Ito, H. and Hirai, Y. (2004). Antibacterial activity of hydrolysable tannins derived from medicinal plants against *Helicobacter pylori*. *Microbiology and Immunology* 48(4) 251 – 261.

- Gill, L. s (1992). *Ethnomedical Uses of Plants in Niger*. University of Benin Press, Benin city, Nigeria.
- Harbone, J.B (1984). *Phytochemical Methods: a guide to Modern Techniques of Plant Analysis* (2<sup>nd</sup> edition). Chapman and Hall, London, pp 19, 37 – 168.
- Ijah, U. J. J and Oyebanji, F. O. (2003). Effects of tannins and polyphenols of some medicinal plants on bacterial agents of urinary tract infections. *Global Journal of Pure and Applied Sciences* 9(2): 193 – 198.
- Ijah, U. J. J., Saidu, S. K., and Uwabujo, A.E. (2003). Identification and antibiograms of bacteria in ear infections in children in Minna, Niger State. *Nigerian Journal of Arts, Sciences and Technology* 1(2): 50 – 56.
- Ijah, U.J.J., and Sar, T. T. (1998). Activities of extracts of medicinal plants on *Klebsiella aerogenes* and *Proteus mirabilis*. *Proceedings of the 11<sup>th</sup> annual National Conference of Nigerian Association of Teachers of Technology*, pp 255 – 258.
- Irobi, O.N. and Daramola, S.O (1993). Antifungal activities of crude extract of *Mitracarpus villosus*. *Journal of Ethnopharmacology* 40: 137 – 140.
- Manna, A. and Abalaka, M. E (2000). Preliminary screening of the various extracts of *Physalis angulata* (L.) for antimicrobial activities. *Spectrum journal* 7(2): 119 – 125.
- Manna. A, Abalaka, A.E. and Garba, S.A (1997). The antimicrobial activity of the leaf extracts of *Calotropis procera*. *Biomedical Letters* 55:205 – 210.
- Martinez, C. M. U., Bentencourt, J., Alenso- Gozalez, N. and Jaourequi, A. (1996). Screening of some Cuban medicinal plants for antimicrobial activity. *Journal of Ethnopharmacology* 52(3): 171 -174.
- Onyeagba, R. A., Ugbogu, O.C., Okeke, C.U and Iroakasi, O. (2004). Studies on the antimicrobial effects of garlic (*Allium sativum* Linn), *African journal of Biotechnology* 3(10):552 – 554.
- Omojasola, P.E and Awe, S (2004). The antibacterial activity of the leaf extracts of *Anacardium occidentale* and *Gossypium hirsutum* against selected microorganisms. *Bioscience Resource communications* 16 (1):25 -28.
- Precott, L.M.,Harley., J.P. and Kleen, D.A(1990). *Microbiology*. WnC Brown publishers, Dubuque, pp. 253 – 255.
- Saxena, R. C(1990). *The Neem Plant: Business opportunities*. Conference on Opportunities in Environmental Conservation and Rehabilitation. Manila, pp. 1 – 17.
- Takeid, Y. and Fatope, M. O. (1998). New phenolic glycoside from *Lowsonia inermis*. *Journal of Natural Products*.51: 725 – 729.
- Trease, G. E and Evans, W.C. (1989). *Pharmacognosy* (13<sup>th</sup> edition). English Language Book Society, Baillaiere Tindall, Britain, pp. 378, 386 - 480.