



An appraisal of the potency of roots of *Anogeissus leiocarpus* (DC.) Guill. & Perr. and *Terminalia glaucescens* Benth. in the management of *E. coli* related infections

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ABSTRACT

Objective: This study analysed the roots of *Anogeissus leiocarpus* and *Terminalia glaucescens* for their chemical constituents and investigated their therapeutic potential in *Escherichia coli* related infections with a view to combating resistant strains and providing basis for future pharmacological research on the two plants.

Methodology and results: The phytochemical analysis of the powdered plant samples was done using qualitative technique. The water and ethanol extracts of the two plants were prepared using cold extraction method. An extract concentration of 10 mg/ml was employed for the antibacterial screening using agar-well diffusion method. The test organisms were clinical isolates of *E. coli* obtained from the University College Hospital (UCH), Ibadan, Nigeria. All data were statistically analysed. The plants contained alkaloids, saponins, tannins, phenols and glycosides. Seventy (70) % of the test organisms were susceptible to the water extracts of both plants at 10^{-6} cfu/ml inoculum concentration of isolates. The ethanol extracts of the plants were active against 100 % of the organisms at 10^{-6} cfu/ml.

Conclusion and application of results: The plants have significant therapeutic potential in the management of *E. coli* associated infections. The isolation of active compounds from the two plants and the study of their mode of actions in infections could lead to the discovery of novel phytodrugs that could be useful in combating multi-drug resistant strains of *E. coli*. The roots of the two plants are sold as chewing sticks (for the prevention of oral infections and mouth odour) in Nigeria. This study indicates the possible antibacterial activity of the plants against oral microbes hence they could be useful in the prevention of tooth decay, gum and throat infections. In addition, the antioxidant screening of the plants could form basis for the assessment of their therapeutic potential in the management of metabolic diseases such as diabetes. The roots of the two plants are commonly used in ethnomedicine in Nigeria; therefore efforts should be directed at their sustainable use via conservation.

Keywords: *Anogeissus leiocarpus*, *Terminalia glaucescens*, herbal recipe, *Escherichia coli*, antibacterial activity, phytochemical analysis.

INTRODUCTION

Anogeissus leiocarpus (African birch) and *Terminalia glaucescens* (Bedda nut tree) belong to family

Combretaceae. The root of the two plants in combination (1:1) are sold as a recipe in herbal

markets in Ibadan, Nigeria and is commonly used in the management of gastrointestinal diseases and lower back pain. Both plants are also sold as chewing sticks for the prevention or treatment of oral infections in southwest Nigeria. In folk medicine, *A. leiocarpus* is used for the treatment of skin infections, wounds, mouth infections, parasitic infection such as malaria, as well as jaundice (Andary *et al.*, 2005). The decoction of young leaves and the bark of *T. glaucescens* are used for the treatment of stomach ache and abdominal pains. It is a valuable plant in the management of malaria; diarrhoea and tooth decay (Ojo *et al.*, 2006). The stem bark and root are used as laxatives. The fruits are used as vermifuge i.e. it expels intestinal worms. The leaf and bark find application in the treatment of naso-pharyngeal infections. The roots are used as medicines for the management of diarrhoea, dysentery; genital stimulants, depressants; leprosy and liver disorder. Also, the roots are used as pain-killers, and for the treatment of skin eruptions and venereal diseases (Burkill, 1985). The potential of *T. glaucescens* in the management of dental and oral infections has been reported (Okunade *et al.*, 2007; Ogundiya *et al.*, 2008; Adebayo & Ishola, 2009). The plant forms part of traditional recipe for the treatment of diabetes in Nigeria (Sonibare & Gbile, 2008). *Escherichia coli* is a Gram negative bacterium found in the intestinal tract of animals. It is the causative organism of neonatal meningitis; an inflammation of meninges of babies characterised by respiratory

problems, convulsion, nausea and jaundice. It is the major organism implicated in Urinary Tract Infections (UTIs), the symptoms being diarrhoea, fever, recurrent urge to urinate, pains and kidney infections. Some strains of *E. coli* are responsible for pneumonia, gastroenteritis, septicaemia, and haemolytic-uremic syndrome (Neugebauer, 1983). Globally, the resistance of bacteria to conventional drugs is high, particularly in developing countries. *E. coli* has antibiotic resistance genes (Bailey *et al.*, 2010) partly caused by uncontrolled use of antibiotics (Yagupsky, 2006; WHO, 2011). Its resistance to fluoroquinolones and cephalosporins is fast increasing in the community setting (Mesa *et al.*, 2006; Laupland *et al.*, 2008). Bacterial fluoroquinolone resistance has been reported in both humans and animals (Hordijk *et al.*, 2011). In view of increasing resistance of pathogenic bacteria to antibiotics, there has been a renewed interest in plant extracts, oils and compounds as antiseptics and antimicrobial agents in medicine (Ahmad & Beg, 2001; Khan *et al.*, 2009; Sekeroglu *et al.*, 2007; Raj *et al.*, 2008). This study examined the chemical components and antibacterial activity of roots of *A. leiocarpus* and *T. glaucescens* against ten clinical isolates of *E. coli* with a view to providing information on the therapeutic potentials of the two plants in the management of *E. coli* related infections, as well as providing basis for future pharmacological research on the two plants.

MATERIALS AND METHODS

Ethnobotanical information: The ethnobotanical investigation revealed that the roots of *A. leiocarpus* and *T. glaucescens* are combined in equal proportions and sold as a recipe in herbal markets in Ibadan, Nigeria. The herbal recipe is used for the management and treatment of diarrhoea, dysentery, stomach ache, lower abdominal pain, pile and lower back pain.

Collection and identification of plants: Fresh and healthy plant-parts of *A. leiocarpus* and *T. glaucescens* were collected from Idi-ayunre in Ibadan, Nigeria. The plants were identified at species level in the University of Ibadan Herbarium (UIH). The plant specimens were prepared and deposited at UIH.

Preparation of powdered plant materials: The test plants were washed, cut into small pieces and completely

dried at room temperature (27°C) for two weeks. The dry plant materials were ground into powder and stored in airtight glass bottles at room temperature prior to experiments.

Phytochemical analysis of plant samples: The powdered plant materials were screened for the presence of phytochemicals using qualitative method (AOAC, 2005) in the laboratory of the Department of Pharmacognosy, University of Ibadan.

Preparation of extracts: Water extract: Powdered plant material (400g) of each plant was shaken in sterile distilled (1000 ml) water for 24 h. The extract was filtered (Whatman No 1 filter paper) and freeze dried to complete dryness. Hundred (100) mg of the extract was dissolved in 10 ml of sterile distilled water to give an extract

concentration of 10 mg/ml which was used for the antibacterial bioassay.

Ethanol extract: Powdered plant material (500g) of each plant was extracted in 80% ethanol (1000 ml) for 48 h. The extract was filtered (Whatman No 1 filter paper) and evaporated to dryness at 40°C using a rotary evaporator. Ten (10) mg/ml of the extract was used for the antibacterial bioassay.

Source and maintenance of test organisms: The test organisms (Table 1) were 10 clinical isolates of *E. coli* obtained through due process from the Medical Microbiology Laboratory, University College Hospital (UCH), Ibadan, Nigeria. The isolates were maintained on nutrient broth (Difco Laboratories, USA).

Table 1: Clinical isolates of *Escherichia coli* used in this study

Isolate code	Source	Diagnosis?
EC1	Blood culture	Sepsis
EC2	Blood culture	Severe head injury
EC3	Endocervical swab	Antepartum haemorrhoid
EC4	Blood culture	Chest trauma
EC5	Ear swab	Otitis
EC6	Urine	Urinary tract infection
EC7	Urine	Second degree infertility
EC8	Urine	First degree infertility
EC9	Urine	Urinary tract infection
EC10	Urine	Urinary tract infection

Antibacterial assay of plant extracts: The agar-well diffusion method was used for the antibacterial screening. The isolates were grown in nutrient broth for 18 h at 37°C. Six concentrations of each isolate of *E. coli* were prepared from the broth in sterile distilled water to give a range of concentrations at 1×10^{-1} to 1×10^{-6} cfu/ml via serial dilution method prior to use. 1ml of the inoculum was thoroughly mixed with 19 ml of sterile nutrient agar and poured into sterile Petri dish. The agar was left to solidify. Two wells of 6 mm in diameter were punctured in each agar plate and 60 µl of each extract was filled into the wells with the aid of a sterile micropipette. Sterile distilled water was used instead of extract in the control

experiment. Also plates containing the test organisms in agar without extract were used as control. All experiments were done aseptically and each experiment was replicated three times. The plates were incubated at 37°C for 36 - 48 h. Readings were taken after 36 h and 48 h. The zone of inhibition was measured in millimetres (mm). **Statistical analysis of data:** All data were statistically analysed using One-way Analysis of Variance (ANOVA) and expressed as mean \pm SD. The Duncan Multiple Range Test (DMRT) was used to test means for significance ($P < 0.05$).

RESULTS AND DISCUSSION

The two plants contained alkaloids, saponins, tannins, phenols and glycosides (Table 2). The phytochemical constituents of *T. glaucescens* have been reported by previous authors (Adebayo & Ishola, 2009; Oshomoh &

Idu, 2011). *T. glaucescens* contains tannins and astringents (Burkill, 1985). The therapeutic value of the two plants in folk medicine might be attributed to their tannin content.

Table 2: Phytochemical constituents of *Anogeissus leiocarpus* and *Terminalia glaucescens*

Plant sample	Alkaloids	Saponins	Tannins	Phenols	Glycosides
<i>A. leiocarpus</i>	+	+	+	+	+
<i>T. glaucescens</i>	+	+	+	+	+

+ = present

The antibacterial activity of water extracts of the plant samples is presented in Table 3. The *E. coli* isolates EC1, EC9 and EC10 were not susceptible to the water extracts

of the two plants. Also not susceptible was isolate EC7 at 10^{-1} cfu/ml inoculum concentration. The highest inhibitory activity of water extracts was observed for *T. glaucescens*

(33.40 mm) against isolates EC4 and EC6, and the least (23.90 mm) was for *A. leiocarpus* also against EC6 at 10^{-1} cfu/ml. *T. glaucescens* had the highest (33.90 mm) activity against EC2 at 10^{-3} cfu/ml and *A. leiocarpus* (9.90 mm) the least against EC7. The highest (34.40 mm)

activity at 10^{-5} cfu/ml was observed for *T. glaucescens* against EC2 and the least (19.40 mm) against EC8 was for the same plant. Overall, EC2 was the most susceptible to water extract of *T. glaucescens* (10^{-3} and 10^{-5} cfu/ml).

Table 3: Inhibitory activity of water extracts of *Anogeissus leiocarpus* and *Terminalia glaucescens* against ten clinical isolates of *Escherichia coli*

Isolate Code	Plant extract	Inoculum load (cfu/ml) / zone of inhibition (mm)		
		10^{-1}	10^{-3}	10^{-5}
EC1	A	0.00 ^j ± 0.00	0.00 ^k ± 0.00	0.00 ⁿ ± 0.00
	B	0.00 ^j ± 0.00	0.00 ^k ± 0.00	0.00 ⁿ ± 0.00
EC2	A	32.90 ^b ± 0.14	23.40 ^d ± 0.14	28.40 ^d ± 0.14
	B	31.40 ^c ± 0.14	33.90 ^a ± 0.14	34.40 ^a ± 0.14
EC3	A	24.90 ^h ± 0.14	23.40 ^h ± 0.14	24.90 ⁱ ± 0.14
	B	32.90 ^b ± 0.14	24.90 ^f ± 0.14	22.40 ^k ± 0.14
EC4	A	24.90 ^h ± 0.14	23.90 ^g ± 0.14	21.90 ^l ± 0.14
	B	33.40 ^a ± 0.14	28.40 ^c ± 0.14	26.90 ^g ± 0.14
EC5	A	29.40 ^e ± 0.56	27.90 ^d ± 0.14	27.40 ^f ± 0.14
	B	30.40 ^d ± 0.14	32.40 ^b ± 0.56	25.40 ⁱ ± 0.14
EC6	A	23.90 ⁱ ± 0.14	23.40 ^h ± 0.14	26.40 ^h ± 0.14
	B	33.40 ^a ± 0.14	23.90 ^g ± 0.14	29.40 ^c ± 0.14
EC7	A	0.00 ^j ± 0.00	9.90 ^j ± 0.14	27.90 ^e ± 0.14
	B	0.00 ^j ± 0.00	14.40 ^e ± 0.14	33.90 ^b ± 0.14
EC8	A	27.40 ^g ± 0.14	23.90 ^g ± 0.14	26.90 ^g ± 0.14
	B	28.90 ^f ± 0.14	26.40 ^e ± 0.14	19.40 ^m ± 0.14
EC9	A	0.00 ^j ± 0.00	0.00 ^k ± 0.00	0.00 ⁿ ± 0.00
	B	0.00 ^j ± 0.00	0.00 ^k ± 0.00	0.00 ⁿ ± 0.00
EC10	A	0.00 ^j ± 0.00	0.00 ^k ± 0.00	0.00 ⁿ ± 0.00
	B	0.00 ^j ± 0.00	0.00 ^k ± 0.00	0.00 ⁿ ± 0.00

*Value = Mean ± standard deviation. Values within a column followed by the same superscript are not significantly different at $P < 0.05$. Diameter of the cork borer = 6.0 mm. A = *Anogeissus leiocarpus* extract. B = *Terminalia glaucescens* extract.

In Table 4, all isolates were susceptible to the ethanol extracts of the two plants at all inoculum concentrations. The highest antibacterial activity was observed for *T. glaucescens* (30.90 mm) against EC2 and the least (17.90 mm) was for *A. leiocarpus* against EC4 at 10^{-1} cfu/ml of all isolates. The highest (36.90 mm) antibacterial effect was observed for *T. glaucescens* against EC7 and

the least (19.90 mm) for *A. leiocarpus* against EC8 at 10^{-3} cfu/ml of all isolates. At 10^{-5} cfu/ml, *T. glaucescens* (36.40 mm) was most active against EC10 and the least (19.90 mm) activity was from *A. leiocarpus* against EC2 and EC4. Generally, isolate EC7 was the most susceptible to the ethanol extract of *T. glaucescens* at high inoculums concentrations (10^{-1} and 10^{-3} cfu/ml).

Table 4: Inhibitory activity of ethanol extracts of *Anogeissus leiocarpus* and *Terminalia glaucescens* against ten clinical isolates of *Escherichia coli*

Isolate Code	Plant extract	Inoculum load (cfu/ml) / zone of inhibition (mm)		
		10 ⁻¹	10 ⁻³	10 ⁻⁵
EC1	A	26.40 ^{bcd} ± 0.14	28.90 ^c ± 0.14	22.40 ^m ± 0.14
	B	24.40 ^{ef} ± 0.14	21.90 ^k ± 0.14	35.90 ^b ± 0.14
EC2	A	20.40 ^{ghi} ± 0.14	22.90 ⁱ ± 0.14	19.90 ^q ± 0.14
	B	30.90 ^a ± 6.92	27.90 ^e ± 0.14	28.40 ^g ± 0.14
EC3	A	28.40 ^{abcd} ± 0.14	22.90 ⁱ ± 0.14	20.40 ^p ± 0.14
	B	23.40 ^{efgh} ± 0.14	22.40 ⁱ ± 0.14	21.90 ⁿ ± 0.14
EC4	A	17.90 ⁱ ± 0.14	24.90 ^h ± 0.14	19.90 ^q ± 0.14
	B	23.90 ^{efg} ± 0.14	20.90 ^m ± 0.14	20.40 ^p ± 0.14
EC5	A	26.40 ^{bcd} ± 0.14	21.40 ⁱ ± 0.14	26.40 ⁱ ± 0.14
	B	29.90 ^{ab} ± 0.14	26.40 ^f ± 0.14	28.40 ^g ± 0.14
EC6	A	24.90 ^{efd} ± 0.14	25.40 ^g ± 0.14	29.40 ^f ± 0.14
	B	22.40 ^{fgh} ± 0.14	28.40 ^d ± 0.14	25.40 ⁱ ± 0.14
EC7	A	26.40 ^{bcd} ± 0.14	31.40 ^b ± 0.14	31.40 ^d ± 0.14
	B	30.40 ^a ± 0.14	36.90 ^a ± 0.14	32.40 ^c ± 0.14
EC8	A	23.90 ^{efg} ± 0.14	19.90 ^o ± 0.14	21.40 ^o ± 0.14
	B	25.90 ^{cdef} ± 0.14	28.40 ^d ± 0.14	24.90 ^k ± 0.14
EC9	A	25.90 ^{cdef} ± 0.14	20.40 ⁿ ± 0.14	22.90 ^j ± 0.14
	B	19.90 ^{hi} ± 0.14	28.40 ^d ± 0.14	27.40 ^h ± 0.14
EC10	A	29.40 ^{abc} ± 0.14	27.90 ^e ± 0.14	30.40 ^e ± 0.14
	B	28.40 ^{abcd} ± 0.14	28.90 ^c ± 0.14	36.40 ^a ± 0.14

*Value = Mean ± standard deviation. Values within a column followed by the same superscript are not significantly different at P < 0.05. Diameter of the cork borer = 6.0 mm. A = *Anogeissus leiocarpus* extract. B = *Terminalia glaucescens* extract.

Fig. 1 shows the comparative antibacterial activity of water extracts of *A. leiocarpus* and *T. glaucescens* against all isolates at 10⁻⁶ cfu/ml. The extracts of the two plants were inactive on EC1, EC9 and EC10. Of the remaining isolates (7), *A. leiocarpus* had higher activity against 5 isolates (EC3, EC4, EC5, EC7 and EC8). *A. leiocarpus* is traditionally acclaimed to be effective in treating infectious diseases in man and animals (Dweek, 1996). Overall, the water extract of *A. leiocarpus* was more active than that of *T. glaucescens* at 10⁻⁶ cfu/ml. The antibacterial activity of the ethanol extracts of the two plants against all isolates at 10⁻⁶ cfu/ml is presented in

Fig. 2. *T. glaucescens* showed higher activity against all isolates than *A. leiocarpus*. The finding of this study on antibacterial activity of *T. glaucescens* is in line with the reports of previous authors. The extracts of *T. glaucescens* demonstrated significant activity against bacterial and fungal isolates; at 100mg/ml, the root extract was more active than the leaf extract against test organisms (*P. aeruginosa*, *B. anthracis*, *S. aureus*, *C. albicans*, *K. pneumoniae*, *E. coli*, *S. typhi* and *Proteus* spp.) (Ayepola, 2009).

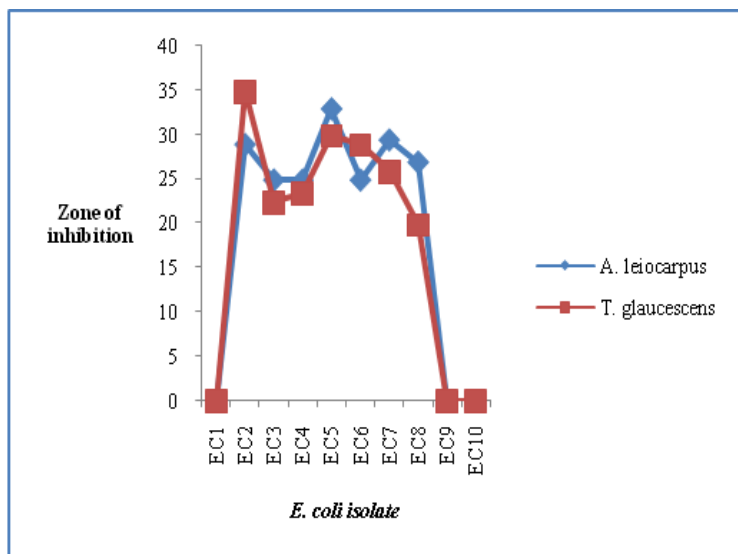


Figure 1: Comparative antibacterial activity of water extracts of *Anogeissus leiocarpus* and *Terminalia glaucescens* against isolates of *E. coli* at 10^{-6} cfu/ml

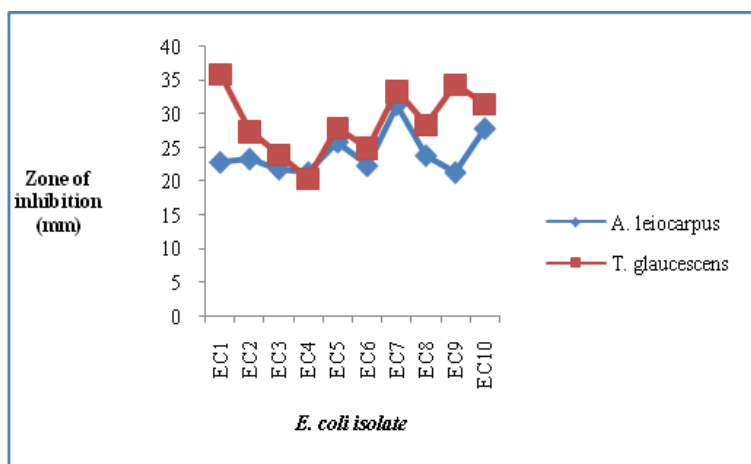


Figure 2: Comparative antibacterial activity of ethanol extracts of *Anogeissus leiocarpus* and *Terminalia glaucescens* against isolates of *E. coli* at 10^{-6} cfu/ml

The methanol extract (10 mg/ml) of *T. glaucescens* roots showed significant inhibitory activity against *E. coli*, it also inhibited the growth of *K. pneumoniae*, *P. mirabilis* and *C albicans* at 60mg/ml (Adebayo & Ishola, 2009). In a study on the antimicrobial activities of the aqueous and ethanol extracts of *T. glaucescens* against dental caries causing microorganisms, Oshomoh and Idu (2011) reported that the stem showed a significantly higher antimicrobial activity against *S. aureus* and *Streptococcus mutans* at a lower concentration of 3.13 mg/ml. The efficacy of the

antimicrobial activities of *T. glaucescens* plant has made both parts (stem and root) suitable for better dental care. There is scarcity of information on the antimicrobial activity of *A. leiocarpus* in the literature. However, Andary *et al.*, [2005] reported that the extracts of the different parts of *A. leiocarpus* showed antimicrobial activity against pathogenic fungi and bacteria. Furthermore, the roots as chewing sticks have broad spectrum antibacterial properties against tooth and gum infections.

CONCLUSION

The phytochemical constituents of *A. leiocarpus* and *T. glaucescens* could be responsible for their therapeutic values in human pathogenic infections. The water and

ethanol extracts of the two plants showed significant antibacterial activity against pathogenic isolates of *E. coli* from clinical sources (blood, urine, ear and endocervical

swab). The isolation of active compounds from the two plants may lead to the discovery of novel drugs that may

be useful in combating resistant strains of *E. coli*.

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