Advances in Natural and Applied Sciences, 3(2): 188-191, 2009
ISSN 1995-0748

ORIGINAL ARTICLE

Evaluation of the Antimicrobial Activity of Root and Leaf Extracts of *Terminalia Glaucescens*

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AYEPOLA O.O: Evaluation of the Antimicrobial Activity of Root and Leaf Extracts of *Terminalia Glaucescens*: *Adv. in Nat. Appl. Sci., 3(2): 188-191, 2009*.

ABSTRACT

An assessment of the antimicrobial activity of the leaf and root extracts of *Terminalia glaucescens* against certain bacterial isolates were carried out. The organisms tested included *Pseudomonas aeruginosa, Bacillus anthracis, Klebsiella pneumoniae, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Candida albicans* and *Proteus spp,* using the agar dilution method. The root and leaf extracts showed appreciable activity against all the tested organisms. However the root extract was found to have a higher activity at 100mg mL^{-1} than the leaf extract at the same concentration especially on the two Gram positive bacteria tested. The mean diameter of the zones of inhibition exhibited by the extracts was between 15mm and 33mm. Minimum inhibitory concentrations (MIC) of the extracts against the bacterial isolates were also determined. The lowest MIC observed for both the root and leaf extracts was 6.25 on *Pseudomonas aeruginosa.* The antimicrobial activity of the extracts was compared with ampicillin used as a positive control.

Key words: Phytochemical screening, antibacterial activity, *Terminalia glaucescens*, agar dilution method.

Introduction

Increases in the emergence of multiple resistant strains of clinically important pathogens have led to the search for more effective antimicrobial agents of plant origin with the aim of discovering potentially active ingredients to serve as a basis for the synthesis of new antimicrobials (Pretorius *et al.,* 2003; Akinsulire *et al*., 2007). *Terminalia glaucescens* a plant indigenous to Africa belongs to the family Combretaceae. In Cameroon, it is traditionally used in the treatment of diabetes (Njomen *et al.,* 2009). It is also one of the widely used plants employed as chewing stick in Nigeria thus various studies have been carried out on its antimicrobial activity against oral pathogens (Ogundiya *et al.,* 2008). Terminalia species as reported by Rahman and Choudhary, (2005) are used in traditional medicines of Central African region. *T. glaucescens* is used in the treatment of dysentery, as an antimicrobial agent and has also found use in the last stages of AIDS (Koudou *et al.,* 1995). Antiplasmodial activity ethanolic extract of the plant was described by Mustofa *et al.,*(2000).

The present study is designed to investigate the antimicrobial activity of the root and leaf extracts of *T. glaucescens* against selected bacteria and a fungal yeast with the aim of providing additional information on the antimicrobial activity of the plant.

Materials and methods

Collection and Extraction of Plant Materials:

The plant materials were collected from Ogbomoso, a town in Oyo State, South West Nigeria. The plant sample was identified by a botanist in the Department of Biological Sciences of Covenant University, where a voucher specimen was deposited.

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The fresh leaves and roots of *T. glaucescens* were sun-dried and finely ground. A weighed quantity of the powdered leaves and roots was extracted with methanol by soxhlet extraction for 48hrs. The extracts were allowed to evaporate to dryness and stored in airtight bottles until ready for use.

Sources and Maintenance of Microorganisms:

Pure cultures of Gram positive organisms (*Staphylococcus aureus, Bacillus anthracis*), Gram negative organisms (*Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Proteus spp, Salmonella typhi)* and a fungal yeast *Candida albicans* were obtained from the research laboratory of the Microbiology Department, Covenant University, Ota, Nigeria. The bacterial strains preserved on nutrient agar at 4^0C were revived in Mueller Hinton broth and incubated overnight at 37^0C. Bacterial suspensions were made to give approximately 10^5cfu/ml.

Antimicrobial Screening of extracts:

The antimicrobial activity of the extracts was determined using the agar well diffusion technique (Adeniyi and Ayepola, 2008) with modification. Mueller Hinton agar plates were seeded with 0.2mL of an overnight culture of each bacterial isolate. The seeded plates were allowed to set and a standard cork borer of 7mm diameter was used to cut uniform wells on the surface of the agar. The wells were then filled with 100µL of each extract at a concentration of 50mg ml^{-1} and 100mg ml^{-1}. Ampicillin at 1mg ml^{-1} was used as positive control. The plates were incubated at 37^0C for 24h after which the diameter of zones of inhibition was measured in millimeters.

Determination of Minimum Inhibitory Concentration (MIC):

The determination of the minimum inhibitory concentration of the extract was carried out using the agar well dilution method as described by Adeniyi and Ayepola (2008).

Different concentrations of the extracts in the range of 50.0, 25.0, 12.5, 6.25, 3.125 and 1.56mg mL^{-1} were prepared. 2mL of each dilution of the extract was mixed with 18mL of Mueller Hinton agar, poured into Petri dishes and allowed to set. The agar was streaked with an overnight broth culture of the bacterial isolates and incubated overnight. The plates were then examined for the presence or absence of growth. In all cases the lowest concentration at which there was no growth was recorded as the MIC.

Results and discussion

The results of the antibacterial activity of the root and leaf extracts of *T. glaucescens* are presented in Table1. The antibacterial activity of the extracts as indicated by the zones of inhibition increased with increase in extract concentration. The diameter of the zones of inhibition ranged between 15mm and 33mm. The root extract at a concentration of 100mg ml^{-1} was observed to have higher activity on all the test organisms than the leaf extract at the same concentration. The leaf extract was active on seven of the eight test bacteria. *Bacillus anthracis* showed the highest sensitivity to all the extracts with the highest zone of inhibition (33mm). Both the root and the leaf extracts had a high activity (30mm) on the fungal isolate *Candida albicans* at 100mg mL^{-1}. The results of the minimum inhibitory concentrations (MICs) presented in Table 2 showed that the root and leaf extracts had the lowest MIC of 6.25mg mL^{-1} on *Pseudomonas aeruginosa.*

Discussion:

The present study revealed that *T. glaucescens* extracts inhibited the growth of almost all test organisms but with varying effectiveness. The root extracts at 100mg mL^{-1} inhibited the growth of all organisms while the leaf extracts at the same concentration inhibited the growth of seven out of the eight test organisms. Higher activity of the root extracts on the tested Gram positive organisms (*Bacillus anthracis* and *Staphylococcus* aureus) than the Gram negative organisms with the exception of *Proteus spp* was also observed. This is because Gram negative bacteria tend to have higher inherent resistance to antimicrobial agents as shown in similar reports by Ndukwe *et al.,* (2005). Ogundiya *et al.,* (2006) also reported a higher antibacterial activity of the root extracts of *T. glaucescens* against *Staphylococcus aureus* and *Streptococcus pyogenes*. *Terminalia glaucescens* is one of the plants used in the preparation of the "wonder cure" concoction used in the treatment of tuberculosis in Nigeria. The activity of the plant extract on *Mycobacterium tuberculosis* was confirmed by Adeleye *et al.,* (2008). The antimicrobial activity of *T. glaucescens* as observed from this study may be

attributed to the presence phytochemicals such as tannins, alkaloids, steroids, anthraquinones, flavonoids and saponins (Ogundiya *et al.,* 2008; Adeleye *et al.,* 2008). The results of the determination of Minimum Inhibitory Concentrations (MICs) showed the root and leaf extracts to have the lowest MIC of 6.25 on *Pseudomonas aeruginosa* an organism known for its intrinsic resistance to various antibiotics and was also found to be resistant to ampicillin used as a positive control in this study.

Table 1: Antimicrobial activities of the root and leaf extracts of *T. glaucescens* at 50mg mL^{-1} and 100mg mL^{-1}

Organisms	TGR 50mg mL^{-1}	TGR 100mg mL^{-1}	TGL 50mg mL^{-1}	TGL 100mg mL^{-1}	Positive control
Pseudomonas aeruginosa	28	30	15	30	-
Bacillus anthracis	30	33	15	25	-
Klebsiella pneumonaie	22	25	-	16	20
E. coli	-	20	-	-	
Salmonella typhi	20	25	20	20	35
Staphylococcus aureus	30	32	15	24	30
Candida albicans	-	30	22	30	-
Proteus spp	30	32	15	22	-

Resistance= no zone of inhibition, Positive control = Ampicillin (1mg mL^{-1})
TGR = *T. glaucescens* root extract, TGL = *T. glaucescens leaf extract*

Table 2: Minimum Inhibitory Concentration (MIC) of the root and leaf extracts of *T. glaucescens*

Organisms	TGR (mg mL^{-1})	TGL (mg mL^{-1})
Pseudomonas aeruginosa	6.25	6.25
Bacillus anthracis	25	50
Klebsiella pneumoniae	50	100
Escherichia coli	25	6.25
Salmonella typhi	25	50
Staphylococcus aureus	25	50
Candida albicans	50	50
Proteus spp	50	50

TGR = *T. glaucescens* root extract, TGL = *T. glaucescens leaf extract*

The results of this study confirms the potential uses of *T. glaucescens* as a source of antibacterial agents against infections caused by *Pseudomonas aeruginosa, Bacillus anthracis, Klebsiella pneumoniae, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Candida albicans* and *Proteus spp.*

Conclusion:

Results from the present study have shown *T. glaucescens* as a potential source of antimicrobial drug against various pathogenic organisms. This is particularly important in combating the recent trend in the emergence of multiple drug resistant organisms. Further studies are however necessary for the development of new antimicrobials from this plant.

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