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Research article

Antibacterial effect of *Terminalia catappa* on some selected pathogenic bacteria

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ABSTRACT

Pathogenic organisms are responsible for the deaths of many people living in both rural and urban areas. However, series of commercial antibiotics have proved ineffective in cure of many of these diseases. Curing of disease(s) is important in health care system and trial of alternative medicine is important in this for good health care delivery. Water extract of the leaf of *Terminalia catappa* at different stages (young, matured and old red pigmented) were studied on some selected pathogenic bacteria species In-Vitro. The leaf extracts at their different stages of maturity, exhibited therapeutic effect on the test organisms. However, the old (red pigmented) leaf extract (C) had inhibitory affinity on the tested organisms with halo ranging between 5mm-10mm in diameter, different from the matured leaf extract (B) that ranged in inhibitory halo of between 10.6mm-18.5 mm. The young leaf extract (A) of *Terminalia catappa* indeed showed the highest inhibitory effect on the test organisms with halos between 10.2- 20.6 mm. Bacillus cereus and Shigella dysenteriae were most inhibited with this extract (20.6 mm) while Escherichia coli was the least inhibited (10.2 mm). MIC was effective by the young leaf extract at 100-145mg/mL, the matured leaf at 130-145mg/mL and the old red leaf extract at 130-350mg/mL on the test organisms. The MBC of the young leaf extract was effective at 100-145mg/mL, the matured leaf extract at 130-160mg/mL and the old red leaf extract at 145-300mg/mL on the test organisms. The antibacterial potency of *Terminalia catappa* is determined so that nutritional and medicinal properties could be exploited judiciously. The results confirm the effective use of this plant in medicine, food system and pharmacy.

Key words: Terminalia catappa, Antibacterial, Pathogenic bacteria, Medicinal plant

1. INTRODUCTION

The treatment and control of diseases by the use of available medicinal plants in a locality will continue to play significant roles in medical health care implications in the developing countries of the world [1]. The antimicrobial activities of plant oils and extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [2]. Moreover, the increasing use of plant extracts in the food, cosmetics and pharmaceutical industries suggests that in order to find active compounds, a systematic study of medicinal plants is very important [3]. According to Azoro [4], before colonialism, herbal medicine was the major form of medicine in Nigeria. However, it was the introduction of orthodox medicine that suppressed the growth and development of herbal medicine in many developing countries. In different part of Nigeria, many varieties of plant species are used in the treatment of different types of diseases. [5], stated that roots, barks or leaves of *newbouldia laevis* are used in the treatment of scrotal elephantiasis, dysentery, ringworm, syphilis, sore eye and ear ache. They also reported that the stem, bark and leaves of *Anthocleista dialonensis* are used as antipyretic and in the treatment of stomach ache, gonorrhoea and fever. [6], reports that shoots of *phyllantus pentandrous* is used in treating boils and back ache. [7] shows that extracts from *Cassia alata* has antimicrobial avtivity against plants which are believed to be the antimicrobial agents and includes alkanoids, sequiterpens, diterpens, tripene saponis, tritepene aglycouts, flavonoinds,

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sterols, coumarin, quinines, monoterpenes, different forms of other proteins as well as lipids and tannies [8, 6, 9].

Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activities. It also facilitates pharmacology studies, leading to the synthesis of a more potent drug with reduced toxicity [10].

Terminalia catappa (tropical almond) is a deciduous tree. The leaves contain agents for chemo-prevention of cancer and anti-carcinogenic potential. Therefore, this work is undertaken to screen the antibacterial effect of the leaves on some selected pathogenic organisms.

2. MATERIALS AND METHODS

2.1 Preparation of plant sample

Apparently fresh and healthy young, matured and old (red pigmented) leaves of *Terminalia catappa* was collected from a compound where it is serving as an ornamental plant. Each portion of the leaves stages in growth were separated washed with water and rinsed in sterile distilled water several times. The leaves were dried at room temperature in the laboratory and homogenized. The obtained powders were extracted with sterile distilled water by soaking for 24 h and filtered. The filtrates were evaporated at 45°C to dried pellets using rotary evaporator (Resona England). The dried extracts were kept in sterile bottles prior use.

2.2 Sources and maintenance of bacterial isolates

The pure isolates of the bacterial used in the study were obtained from the State Specialist Hospital, Akure, Ondo State, Nigeria.the isolates were maintained throughout the period of study at two weeks interval. Prior to use, the isolates were inoculated into separate cotton-plugged test tube containing 10mL Mueller Hinton broth which were inoculated at 37°C for 24 h. The pathogenic organisms used are *Bacillus cereus, Staphylococcus aureus, Psuedomonas aeruginosa, Escherichia coli, Proteus mirabilis, Salmonella typhi and Shigella dysenteriae*.

2.3 Preliminary screening of secondary metabolites

This aspect was determined using chemical methods and by adopting standard protocols to identify the constituents as described by [7, 8].

2.4 Quantitative estimation of secondary metabolite

Standard protocols were used. Saponins was determined by the criteria of Obadoni and Ochuko, 2001; flavonoids by the methods of Boham and Kocipai-Abyzazan, 1974; tannins by the criteria of Van-Burden and Robinson, 1981; alkaloids was determined by the methods of Ikan, 1981 and phenols by the methods of Bray and Thorpe, 1964.

2.5 Sensitivity screening method

The use of well-in-agar diffusion method was adopted. The bacterial isolates at concentration of 10^7 cells/ mL in their log phase was pour plated with Mueller Hinton agar. The plates were allowed to stand for 2 h for the test organisms to be fully embedded in the growth medium before a cork borer (No 4) was flamed and used to bore wells. The different leaf extracts in their crude forms were filled in the dug wells and labelled appropriately. The plates were incubated at 37°C for 24 h. The sensitivity of the test organisms to the extracts was evaluated by measuring the inhibitory halo with a transparent plastic rubber and was taken as an index of the degree of sensitivity.

2.6 Minimal inhibitory concentration

The minimum inhibitory concentration (MIC) of the extracts was determined by broth dilution method. Decreased concentrations of the extract were prepared (400-100mg/mL). The extracts were weighed and reconstituted appropriately in sterile distilled water. In each test tube containing 8mL of sterile Mueller Hinton broth, 1mL of the different extract concentration, and 1mL overnight broth culture of the test organisms were introduced. The tubes were rolled between the palms for even mixed up and incubated at 37°C for 24 h. Turbid tubes after incubation indicates negative and the least extract concentrations where clarity in medium is visible to the naked eyes, determined the MIC of the extracts.

2.7 Minimal bactericidal concentration

Minimal bactericidal concentration (MBC) was determined by plating 1mL of the MIC positive tubes on nutrient agar to ascertain its bacteriostatic and bactericidal effect of the leaf extracts.

2.8 Conventional antibiotic disc assay

The oxoid laboratory multor sensitivity disc (Gram -ve and Gram + ve) was used to assay the sensitivity pattern of the test organisms in comparism to the leave extracts. The antibiotics and concentrations impregnated to the disc arms are Augmentin (AUG) 30µg, Amoxylin (AMX) 2µg, Ciprofloxaxin (CPX) 10µg, Gentamycin (GEN) 10µg, Nitrofuration (NIT) 200µg, Oflaxacin (OFL) 54µg, Penfloacin (PFX) 5µg. However, the same method used for the extract assay was also adopted for this test, except that the discs were picked with a sterile forceps and positioned at the centre of the seeded nutrient agar plates.

3. RESULTS AND DISCUSSION

The young leaf extracts of *Terminalia catappa* showed highest inhibitory potential than the matured and old red leaf extracts. *Bacillus cereus* and *shigella dysenteriae* were each

Table 1
Inhibitory assay of *Terminalia catappa* leaf extracts on the test organisms

Test organisms	Inhibitory assay of *Terminalia catappa* extracts (mm)		
	Young leaf	Matured leaf	Old red leaf
Bacillus cereus	20.6	17.0	7.0
Staphylococcus aureus	18.0	16.0	5.0
Pseudomonas aeruginosa	12.4	17.2	6.0
Escherichia coli	10.2	10.2	7.0
Proteus mirabilis	10.6	9.8	8.0
Salmonella typhi	18.3	9.4	8.0
Shigella dysenteriae	20.6	18.5	10.0

Table 2
Extract concentration at which MIC and MBC are valuable on the test organisms

Test organisms	Extract concentrations (mg/mL)								
	Young leaf			Matured leaf			Old red leaf		
	MIC	MBC	Action	MIC	MBC	Action	MIC	MBC	Action
Bacillus cereus	100	100	C	145	160	S	350	145	S
Staphylococcus aureus	100	115	S	130	160	S	300	130	S
Pseudomonas aeruginosa	100	145	S	140	130	S	300	130	S
Escherichia coli	100	100	C	130	130	C	300	300	C
Proteus mirabilis	145	145	C	130	130	C	300	300	C
Salmonella typhi	100	100	C	130	145	S	250	130	S
Shigella dysenteriae	100	100	C	145	145	C	300	300	C

MIC= Minimal inhibitory concentration, MBC= Minimal bactericidal concentration, S= Bacteriostatic, C= Bactericida

Table 3
Conventional antibiotic disc assay (mm)

Test organisms	AUG	NIT	AMX	CRO	COT	CPX	GEN	OFL	PEX	TET
Bacillus cereus	-	6	8	10	-	15	1	3	5	-
Staphylococcus aureus	-	29	20	15	-	28	10	12	15	2
Pseudomonas aeruginosa	-	11	20	15	1	10	2	5	-	-
Escherichia coli	-	-	-	-	-	-	-	-	-	-
Proteus mirabilis	-	7	10	11	8	5	-	10	-	-
Salmonella typhi	-	-	-	-	2	1	-	2	3	-
Shigella dysenteriae	-	15	18	26	26	22	-	13	-	5

AUG: Augmentin, NIT: Nitrofurantoin, AMX: Amoxicillin, CRO: Celtridzone, COT: Contrimoxazole, CPX: Ciprofloxacin, GEN: Gentamycin, OFL: Oflaxacin, PFX: Pefloxacin, TET: Tetracyclin

inhibited with a zone of 20.6 mm. Despite the growth stages of the leaf inhibited all the test organisms with various degrees of inhibition, *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhi* and *Shigella dysenteriae* were the most inhibited. However, it was observed that the inhibitory halo (10.2-18.5 mm) displayed by the matured leaf extract were close to the inhibitory sensitivity (10.2-20.6 mm) displayed by the young leaf extract. The inhibitory potential observed with the red old leaf extract (5-10 mm) was not comparable to the therapeutic effect of young and matured leaf extracts on the test isolates (Table 1).

The phytochemicals qualitatively identified are tannins, saponins, flavonoids, alkaloids and phenol. The quantities of tannins, saponins, flavonoids, alkaloids and phenol in the young leaf extract were found to be 20.11± 0.1, 3.2± 0.1, 0.25± 0.1, 1.53± 1.2 and 0.34± 0.0, respectively. For matured leaf extract, the same phytochemical constituents were, 1.6± 0.1, 0.18± 0.1, 19.36± 0.1, 1.36± 1.2 and 0.28± 0.0 and for old red leaf extract, 0.18±0.1, 0.10±0.1, 10.18±0.1, 1.03±1.2 and 0.20±0.0, respectively. The appreciable quantities of phytochemicals identified in the leaf extracts were responsible for the high antibacterial effect observed. Their inhibitory potency on the tested isolates was as due to the crude form which contained the phytochemicals in large quantities, therefore directional to all kind of infections unlike purified antibiotics which are directional to a type of infection because the phytochemicals are separated into single or double entity for specificity in prevention and curing of diseases.

The MIC of the leaf extracts pointed out 100-145mg/mL, 130-145mg/mL and 200-350mg/mL respectively for the young, matured and red old leaf extracts as the actual therapy value. Comparing the bacterial load of the MIC positive tubes to the initial load (10^7cell/mL) of the test organisms, reduction in load was observed at 100mg/mL concentration. Though clarity were observed with the rated eyes in the

positive MIC tubes, the MBC assay, revealed the concentrations at which the leaf extracts were bacteriostatic and bactericidal on the test organisms. The cidal and static activities of the leaf was in the trend of its antibacterial assay (Table 2). The employed commercial antibiotics (reference drugs) antibacterial activities in some cases showed higher inhibitory potency and in other cases showed lower inhibitory potency than the leaf extracts. NIT, AMX, CRO and CPX were the most potent on the test organisms. *Staphylococcus aureus* was the most inhibited (10-28 mm) by the reference drugs followed by *Shigella dysenteriae* (13-26 mm) among the test bacterial isolates. All the test organisms were resistant to augumetin. However, *E.coli* was resistant to all the employed reference drugs. *S. typhi* was resistant to AUG, NIT, AMX, GRO, GEN and TET. TET showed the least therapeutic effect (2-5 mm) on the test bacterial isolates (Table 3).

It has been reported that Gram negative bacterial are resistant to antibacterial agents and this was observed in the employed reference antibiotics. Hence they are purified agents and the leaf extracts in this study acted better on both the Gram positive and negative considered test organisms, their purification will evident a high therapeutic effect on certain disease(s) in which they are specifically manufactured for. The findings in this study, shows that the crude extract of the different growth stages of *Terminalia catappa* leaves acted significantly as antimicrobial agent. This was demonstrated by the various inhibitory halo measured from the in-vitro bio-assay.

However, the active substances contained in the leaves made it possible for the inhibitory measure over the test organisms. Though, [11, 12, 13] have reported the presence of some compounds such as saponin, glycosides, steroid, cardiac glycosides, tannins, volatile oils, phenols and balsam (gum) in members of the family combretaceae, to which *Terminalia catappa* belong, the quality and quantity of the phytochemicals identified in the study suggests it that the family of combretaceae are rich in secondary metabolites, appreciable in the physiological and pharmacological effects on man and probably animal. Since decreased inhibitory halo was observed in the stages of aging in *Terminalia catappa* leaf, it implies that the active ingredients in the leaf decrease alongside advancement in age. Despite the fact that the leave extracts acted on the test organisms, the satisfactory result obtained on their efficacy was greatly highlighted by the variations in the extracts capability at the leaf different stages, higher inhibitory potency than the employed commercial antibiotic and time of incubation. Though the active ingredients of these leaf extracts are known, the satisfactory therapeutic potency exhibited even with water extraction, indicates that the concentration of the active components contained, is high and easily extractable. The search for more plants with antimicrobial agents should continue, in other to guide against organisms that often evolve into new genetic variants, which may subsequently be resistant to the existing therapeutic agents as seen in the antibiotic resistance activity by some of the test organisms.

4. CONCLUSIONS

This study is vital because the misuse of antibacterial drugs is on the rampage and when an organism is possessed with resistance factors, it will pose a health treat which its control or prevention might not be quickly come by. However, work continues on this leave extracts which in due course may suggest its phytochemical components and other useful information for the possible use of the extract on human beings suffering from any disease that might be manifested by the test organisms.

REFERENCES

[1] Akharaiyi, F.C., Boboye, B., *J Nat Prod* 2010, *3*, 27-34.

[2] Lis-Balchin, M., Deans, S.G., *J Appl Bacteriol* 1997, *82*, 759-762.

[3] Nostro, A., Germano, M.P., D'Angelo, V., Marino, A., Cannatelli, M.A., *Lett Appl Microbiol* 2000, *30*, 379-384.

[4] Azoro, C., *The Nigerian Journal of Research and Production* 2004, *3*, 132-127.

[5] Nwaeze, C.U., Abarikwu, P.O., *Nigerian Journal of Microbiology* 1986, *4*, 32-40.

[6] Otiko, G. *Nigerian Journal of Biothechnology* 1987, *4*, 121-123.

[7] Ikenebome, M.J., Metitiri, P.O., *Nigerian Journal of Microbiology* 1998, *8*, 12-33.

[8] Sofowora, A., *Medicinal Plants and Traditional Medicine in Africa*, Spectrum books ltd, Ibadan 1993, pp. 172-188.

[9] Lambo, O., *The African Medicinal Plant*. University of Ile-Ife Press, Nigeria 1982, pp. 23-24.

[10] Manna, A., Abalaka, M.E., *Spectrum J* 2000, *7*, 119-125.

[11] Ahmad, I., Mahmood, Z., Mohammad, F., *Journal of Ethnopharmacol* 1998, *62*, 183-193.

[12] Pamplona- Roger, G.D., *Encyclopedia of Medicinal Plants*, Vol. 1 and 2, Education and health library. The European Union 1999, pp. 128-150.

[13] Shariff, Z.U., *Modern Herbal Therapy for Common Ailments*, Nature Pharmacy Series (Vol.1). Spectrum books ltd. Ibadan, Nigeria in association with safari books (Export) limited, United Kingdom 2001, pp. 9-84.