Antibacterial and phytochemical effects of plantain (*Musa paradisiaca* L.) and tropical almond (*Terminalia catappa* L.) against clinical isolates

Adomi, Patience O.

Department of Microbiology, Faculty of Science Delta State University, Abraka, Nigeria.
E-mail: padomi.adomi07@gmail.com.

Accepted 16th June, 2021

Musa paradisiaca and *Terminalia catappa* leaves and stembark were studied for antibacterial and phytochemical activities. The water, ethanol and methanol crude extracts of *T. catappa* leaves and stembarks were obtained using soxhlet extraction while the root sap of *M. paradisiaca* was obtained manually under asceptic condition. Antibacterial activity was evaluated by *in vitro* disc diffusion method and phytochemical compounds were detected using standard methods. *T. catappa* ethanolic extract of leave demonstrated the highest activity against *Escherichia coli*. *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus saprophyticus, E. coli* and *Proteus miarbilis* were sensitive to methanol and water extracts of *T. catappa*. No bacterium was sensitive to *Musa paradisiaca* root sap. *T. catappa* leave and stem extracts contained various phytochemical compounds except anthraquinones which was lacking in the stem. *T. catappa* leaves and stem barks could be used as antibacterial agents, in the management of infectious diseases associated with these organisms.

Key words: *Musa paradisiaca*, root sap, *Terminalia catappa* leaves and stembark, antibacterial phytochemical.

INTRODUCTION

Musa paradisiaca L. (Plantain) is a mono herbaceous plant, belonging to the family of Musaceae, native to Southeast Asia and India and cultivated in tropical and subtropical regions of the world. The plant have two genera and 42 different species (Trease and Evans, 2002). Some varieties grows up to 9 m long, with a robust tree like pseudostem, a crown of large elongated oval deep-green leaves, with notable midrib. Each plant make a single inflorescence like drooping spike and large bracts opening in succession, ovate, 15-20 cm long, concave, dark red color and in somewhat fleshy. Fruits are oblong, fleshy, 5-7cm long in wild form and longer in the cultivated varieties (Dutta et al., 1986). Fruit berries in several clusters are picked when they are unripe and starch-rich, but when ripen, (golden yellow colour), the starch turns into simple sugars such as sucrose, glucose, and fructose.

Ethnobotanically, *M. paradisiaca* is used in the treatment of gonorrhoeae, and other non gonococci veneral diseases, inflammation, hypertension, rheumatism, diabetes, and malaria (Odugbemi and Akinlisure, 2006). The unripe bananas and plantain fruits are astringent, and are used to treat diarrhoeae. In addition, combination of *M. paradisiaca* leaves and other medicinal plants are used to cure respiratory diseases. The roots contain antihelmintic and astringent features and stop coughing up of blood (hemoptysis). The juice is used as remedy for snakebite. Asthma, migraine and jaundice are other diseases plant is used to treat. Fruits and flowers are used as mild laxative and adstringent. The medicinal spectrum of the plant also include the management of diarrhoeae and dysentery, intestinal lesions in ulcerative colitis. It is also used to manage celiac disease, constipation and peptic ulcer (Joshi, 2006). The unripe fruit and cooked flower are

useful in treating diabetes. The stems melt preformed stones and prevent the cumulation of stones in the urinary bladder of rats (Gibson, 1998). Honey mixed with root sap is used for the treatment of enlarged prostate (Eseyin et al., 2011).

T. catappa L. is localized in the coastal areas where it grows in subtropical and tropical countries. *T. catappa* is an erect, tall, semi-deciduous tree with average size between 10 to 25 m belonging to Combretaceae family. The common name include Tropical almond, wild almond, India almond, sea almond. It is locally called "Ebelebo". The tree trunk appear cylindrical and straight but can also be thin and twisted with greyish brown coloured bark which gets rough with age. The branches are erect, horizontal, arranged in sequence that make the tree have a pagoda shape that is not usually noticeable since the branches stretch out and drop at the tips. The leaves are large, measuring 1.5 to 3.6 m by 8-2.4 m, single alternate with ovate shape and cluster spirally around the tips, dark green in colour, and looking pale below, shiny, and leathery at the surface. Before detaching from tree, the leaves colour changes to yellowish red. The flowers are small, white or cream coloured, five lobed and arrayed or positioned on long auxiliary spikes. There are no petals, commonly, male and bisexually located at base of spikes. Fruits are rigid, compressed laterally, oval-shaped and drupe. The fruit is reddish purple in full maturity but green when young. The rind has pithy tissue and light in weight as such can float and dispersed by water. Each fruit contain cream coloured seed, which encircle the kernel (nut) (Saini et al., 2010).

In folk medicine, hot water infusion of the fallen leaves, after drying and shredding is used for high blood pressure, urinary infections, gastritis and cardiac weakness (Giron et al., 1991).

In Guatemala, *T. catappa* is useful in the treatment of sexual dysfunction. In Papua New Guinea, fresh sap is employed for cough while decoction of dried bark is applied on sores (Holdsworth, 1984), in addition, fresh bark infusion is employed as emetic, abortifacient, amenorrhea and malaise (McClatchey, 1996). Juice from leaves is used for diabetes in Fiji (Singh, 1986) and colic due to indigestion in Cameroon (Noumi and Yomi, 2001). Haemoptysis gonorrhoeae, ulcers, cough, catarrh, cholagogues and other ailments. Astringent, cardiac tonic, styptic, emollient and diuretic are other uses of *T. catappa* (Odugbemi and Akinsulire, 2006). The antibacterial activities of *T. catappa* crude extracts and *M. paradisiaca* root sap were determined to verify the claims of their uses in traditional medicine.

MATERIALS AND METHODS

Plant collection and extraction

The leaves and stem barks of *T. catappa* and *M. paradisiaca* root sap were collected from the wild from Abraka, Delta State. The plants were identified and their voucher numbers were UBH-T541 and UBH-P540 respectively. The plants were dried under room temperature for three weeks. *T. catappa*, extracts were obtained using soxhlet extractor and using a modified method of Neelavathi, et al. (2013). The extracts obtained were concentrated using rotary evaporator (Model- R/180Buchi Labortech, Switzerland) then dried to a constant weight in the oven. The solid crystal/oily extract obtained and weighed was then kept in sterile labelled air tight bottle and stored at +4°C. The root sap of *M. paradisiaca* was obtained by harvesting the root part of the plant, washed and cut into small pieces. These were crushed with a sterile mortar and pestle. The juice was pressed out manually and filtered with a sterile muslin cloth. The juice was kept until used same day.

Antibacterial tests of crude extracts

Antibacterial activities of the test plant extracts were carried out by adopting modified method of Bauer et al. (1966) for the sensitivity testing. The organisms used for this study were bacterial isolates from previous study (Adomi, 2019). They included *Escherichia coli, Staphylococcus aureus, Staphylococcus saprophyticus, Proteus mirabilis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* The organisms were resursitated by growing in Mueller hinton broth. Filter paper disc were prepared by cutting Whatman No.1 (6 mm) using a perforator, then sterilized using hot air oven (Venkatalakishmi and Brindha, 2016). Extracts (1000 mg) were dissolved in 4 ml of

solvents to make 250 mg/ml. The juice of *M. paradisiaca* was used without further dilution, 200 µl of appropriate concentration of extract was introduced into sterile disc. Mueller hinton agar prepared according to manufacturers instructions were inoculated with standardized bacteria and the disc were placed on the seeded plate. Inoculum was standardized according to description of Cheesbrough (2004). Actively growing cell in broth culture was diluted further with sterile broth and compared with 0.5 McFarland standard. The plates were incubation at 37°C for 24 h. Controls discs were prepared using solvents (water, ethanol and methanol) used for plant extraction. Three plates each for a dilution were inoculated.

Statistical analysis

The inhibition zone data were expressed as means ± standard deviation. The data were subjected to ANOVA, followed by Duncan's post hoc test to evaluate significant differences among the groups of treatments. All significant tests were at $P<0.05$ levels and all analysis was done using SPSS 22 (SPSS, Inc., USA).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC for extracts were determined by broth dilution method (CLSI, 2016). The concentrations of extracts used was within the range of 3.91-250 mg/ml. All experiments were conducted in dublicates. Standardized bacterial cell suspension (0.12ml) was inoculated into each tube and incubated at 37°C for 24 h a clear tube with the lowest concentration of extracts was taken as the MIC. Controls included broth only; broth and sterile plant extract; and broth with a test organism. While MBC was determined by inoculating the last tube with growth and others that did not grow into agar. The clear test tube (not turbid) having the lowest concentration of extracts that did not show growth on agar at the end of incubation period was the MBC.

Phytochemistry screening

Phytochemical tests were carried out using methods of Odebiyi and Sofowora (1978) and Harborne (1973). Alkaloids, reducing sugars, saponins, anthraquinone, phlobatannins, tannins, terpenoids and steroids were determined for *T. catappa* leaves and stem bark.

RESULTS AND DISCUSSION

The study investigated the antibacterial and phytochemical components of *M. paradisiaca* root sap and *T. catappa* leaves and stem bark extracts against clinical bacterial Isolates. The percentage yield of crude extracts of *T. catappa* is shown in Table 1. The highest yield was 6.30 g for water stem extract and the lowest yield was 1.5 g for methanol extract. Findings showed that the root sap from *M. paradisiaca* was not active against any bacteria (Table 2). As at the time of this study, no work on *M. paradisiaca* root sap has been reported however methanol and ethanol extracts of peels and fruits had been reported to be active against the test bacterial isolates with minimum inhibitory concentration range of 100-200 and MBC 200-300 mg/ml (Asoso et al., 2016).Tables 3-5 show the effect of *T. catappa* crude extracts on the isolates. *Pseudomonas aeruginosa* had the highest zone of inhibition of 16.33 ±0.88 mm for leaf extract at 250 mg/ml, while *E. coli S. aueus, S saprophyticus* showed varied sensitivities to crude extracts. Only three bacterial isolates were susceptible to methanol extracts. The results for each concentrations were 7.33±0.30, 8.00±0.58, 10.00±0.58 and 12.00±0.58 mm respectively for *S. saprophyticus*. Significant increase was observed for the various zones of inhibitions when compared with those taken for the lowest concentration (32.25 mg/ml) (Table 4). Ethanol extract was the most potent, all the test bacteria in this study were susceptible to this extract compared to other extracts. *E. coli, S. aureus, K. pneumoniae, S. saprophyticus* and *P. mirabilis* were sensitive to the leaf extract. The results obtained in this study are similar to previous study, where the leaves of *T. catappa* were reported to be active against bacteria and fungi. In that study, Gram positive bacteria were more sensitive to the leaf extract than gram negative bacteria (Manzur et al., 2011). However, in this study, the most susceptible organism was *E. coli* having the highest zone of inhibitions of 21.31± 1.45mm. As noted earlier, zone of inhibition recorded increased significantly when compared to that from first

Table 1. Percentage yield of plants extracts of *T. catappa* leaf and stembark extacts.

Plants	Water(%)W/V	Ethano(l%)W/V	Methanol(%)W/V
Terminalia catappa L. (stem)	6.30	5.20	3.80
T. catappa L. (leaf)	5.60	4.40	1.50

Table 2. Result showing the antibacterial effects of *Musa paradisiaca* root juice against clinical isolates.

Plant extract	*Staphylococcus saprophyticus*	*Proteus mirabilis*	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
M.paradisiaca root sap	ND	ND	ND	ND	ND	ND

Key :ND= No inhibition detected.

Table 3. Effect of *Terminalia catappa* water extracts on clinical isolates.

Bacteria		Concentration of Crude extracts (Mg/mL)			
		31.25	62.50	125	250
Escherichia coli	Stem	9.00±0.58	10.33±0.58	12.00±0.00*	13.00±0.00*
Staphylococcus aureus	Stem	8.00±0.58	9.00±0.00	12.00±0.58	14.00±0.58*
Staphylococcus saprophyticus	Stem	7.00±0.00	8.00±0.58	10.00±0.58	13.00±0.58*
Pseudomonas aeruginosa	Leaf	0.00±0.00	0.00±0.00	11.33±0.60*	16.33±0.88*

Value are expressed as mean ± STD * values are significantly (P>0.05) compared to 31.25 Mg/ml

Table 4. Effect of *T. catappa* Methanol extracts on clinical isolates.

Bacteria		Concentration of Crude extracts (Mg/ml)			
		31.25	62.50	125	250
Stahylococcus saprohyticus	Stem	7.33±0.30	8.00±0.58	10.00±0.58	12.00±0.58*
Proteus mirabilis	Leaf	6.00±0.58	8.00±0.58	10.00±0.58*	11.67±0.92*
Proteus mirabilis	Stem	8.00±0.58	8.00±0.58*	9.00±0.29*	13.00±0.29*

Value are expressed as mean ± STD * values are significantly (P>0.05) compared to 31.25 Mg/ml.

Table 5. Effect of *Terminalia catappa* Ethanol extracts on clinical isolates.

Bacteria		Concentration of Crude extracts (Mg/mL)			
		31.25	62.50	125	250
Escherichia coli	Leaf	11.00±0.00	16.33±0.33*	16.67±0.33*	21.33±1.45*
Escherichia coli	Stem	8.00±0.29	12.00±0.58*	15.00±0.00*	15.67±0.67*
Staphylococcus aureus	Leaf	6. 00±0.58	7.00±0.00	9. 00±0.58*	10..67±0.88*
Staphylococcus aureus	Stem	7.33±0.68	10.00±0.00*	12.00±0.58*	15.33±0.88*
Staphylococcus saprophyticus	Leaf	7.00±0.58	9.00±0.00*	10.00±0.58*	11.67±0.88*
Klebsiella pneumoniae	Leaf	9.00±0.00	11..00±0.88	10.007±0.67	11.00±0.58
Proteus mirabilis	leaf	8.00±0.58	9.00±0.00	10.0±0.00	13.33±1.15*

Value are expressed as mean ± STD * values are significantly (P>0.05) compared to 31.25 Mg/ml.

concentration (32.25 mg/ml). Stem ethanol extract was active against *E. coli* and *S. aureus*. (Table 5). Minimum inhibitory and bactericidal concentrations of *T. catappa* is shown in Table 6. Minimum inhibitory concentration ranged from 62.25 to 125mg/ml while the MBC ranged from 125-250 mg/ml. From this study, *T. catappa* leaf and stembark showed presence of flavonoids, phlobatannins, cardiac glycosides, anthraquinones, steroids, and terpenoid (Table 7). The

Table 6. Minimum Inhibitory/bactericidal concentration of crude extracts.

Bacteria	Minimum inhiitory concentration (Mg/ml)			Minimum bactericidal concentration (Mg/ml)		
	Water	Methanol	Ethanol	Water	Methanol	Ethanol
	Leaf/Stembark	Leaf/Stembark	Leaf/Stembark	Leaf/Stembark	Leaf/Stembark	Leaf/Stembark
Escherichia coli	-/125	-/-	62.25/125	-/250	-/-	125/250
Staphylococcus aureus	-/125	-/-	125/125	-/250	-/-	250/250
Staphylococcus saprophyticus	-/125	-/125	125/-	-/250	-/250	250/-
Klebsiella pneumonia	-/-	-/-	125/-	-/-	-/-	250/-
Proteus mirabilis	-/-	125/125	125/-	-/-	250/250	250/-
Pseudomonas aeruginosa	125/125	-/-	-/-	125/125	-/-	-/-

Table 7. Results of qualitative tests of *Terminalia catappa* leaf and stem extracts.

Phytochemical tests	leaf	Stembark
Alkaloids	+	+
Reducing sugars	+	+
Saponins	+	+
Cardiac glycosides	+	+
Anthraquinones	+	-
Phlobatanins	+	+
Tannins	+	+
Flavonoids	+	+
Terpenoids	+	+
Steroids	+	+

Key:-= negative, += positive.

phytochemical results however contrasted result from another study on *T. catappa* leaves which showed presence of flavonoids, quinones, phenolics, triterpenoids and tannins and absence of alkaloids, steroids and saponins (Allyn et al., 2019). The study revealed that *M. paradisiaca* root sap was not active against the test bacteria isolates studied however, *T. catappa* leaves and stem extracts were active. Ethanol extract was the most active than water and methanol extracts. *T. catappa* stem extracts lacked anthraquinones but contained other phytochemical compounds.

CONFLICT OF INTERESTS

The author have not declared any conflict of interests.

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