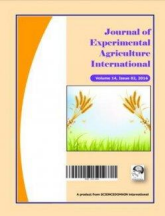

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In vitro Antioxidant Potency and Antifungal Efficiency of Four Local *Terminalia* Species against *Fusarium* Strains

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate *in vitro* antifungal activities of four selected *Terminalia* species and their antioxidant potencies.
Study Design: Activities were directed on the *in vitro* antifungal and antioxidant potency of plant's extracts.
Place and Duration of Study: The study was carried out at the Department of Environment and Plant Protection, and Laboratory of Chemistry and Biochemistry, University of Agricultural Sciences and Veterinary Medicine (USAMV) between March to July 2014, Laboratory of Biochemistry and Microbiology (Bioactives Natural Substances Unit), Jean Lorougnon Guédé University between September 2014 to January 2015.

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Methodology: Growth inhibitory effect of ethanolic and methanolic extracts of the stem barks of *Terminalia Catappa, Terminalia ivorensis, Terminalia mantaly and Terminalia superba* were tested *in vitro* by applying agar slant double dilution method against *Fusarium oxysporum* sp *tuliipae*, *Fusarium oxysporum* sp *radicis-lycopersici* and *Fusarium graminearum*. MIC and MFC values were determined. The measurement of antioxidant activity of the tested extracts was done through DPPH scavenging method.
Results: All test fungi were susceptible to both ethanolic and methanolic extracts of all *Terminalia* species tested in dose-effect relationship. Methanolic extracts of each *Terminalia* sp. tested had lowest values with MIC and MFC ranging between 3.125 to 25 µg/ml. *Terminalia ivorensis* extracts were the most active (MIC ranging between 3.125 to 50 µg/ml) and *F. oxysporum sp tulipae* was the most susceptible fungi to the different plant extracts. Methanolic extracts of each *Terminalia* sp were fungicidal. The highest potentials antioxidant were obtained with *T. ivorensis* extracts at 462.34 mM Trolox/1 ml methanolic extract and 444.4114 mM Trolox/1 ml ethanolic extract.
Conclusion: The stem bark of *Terminalia* species tested had potential as both sources of antifungal compounds for controlling *Fusarium* contamination and natural antioxidant substances for human healthcare.

Keywords: Terminalia species; antifungal activity; antioxidant.

1. INTRODUCTION

Fusarium species are plant pathogenic fungus that causes "Fusariosis" in more than a hundred species of plants such as tomato, maize, sugarcane, Tulip, Banana, etc. They colonise the leaves, roots or seeds of the host plant, and as a result, lead to wilt disease symptoms such as, leaf wilting, yellowing and eventually the death of the plant [1]. Management of *Fusarium* species is required, as this pathogen and its many special forms affect a wide variety of hosts of economic value. The development of resistance to common fungicides and increasing restrictions on the use of toxic material in the environment has given an impetus to the search for novel plant protectants that interfere with the fungal pathogenicity factors and without harm effects on human. Use of natural products for the control of fungal diseases in plants is considered as an alternative to synthetic fungicides, due to their lower negative impacts on the environment. Besides being harmless and non phytotoxic, it has been proved that some plant extracts exhibit good antioxidant activities as well [2,3]. Thus, these plant extract could be also a source of natural antioxidant substances which can allow to fight against oxidative stress [4,5].

Terminalia species belonging to the family Combretaceae, are rich in polyphenolic components and triterpenoids [6,7] and could be species for the development of new natural biofungicides. Different parts of the plants of this genus have long been used as folk medicine in western Africa for antidiarrheic, antipyretic and hemostatic purposes [8,9]. In several countries, researchers have used the leaves, bark, and fruit of some species of *Terminalia* to treat dermatitis and pyresis [10]. Moreover, the anti-inflammatory, hepatoprotective, antidiabetic, carcinogenesis-preventing, antimalarial, antinociceptive and antimicrobial [11,12,13] activities of *Terminalia* species have been studied and this genus could potentially provide benefits to humanity in agricultural domain and human healthcare. In Côte d'Ivoire, it has been reported by earlier researches that *Terminalia catappa* L., *Terminalia* ivorensis A Chev, *Terminalia mantaly* H. Perrier and *Terminalia superba* (Engl. & Diels) were found to possess good antifungal activities against human pathogens by reducing the *in vitro* growth of some yeast and moulds [14].

To reduce the use of chemical fungicides in agriculture, use of natural antimicrobial substances derived from *Terminalia* species would primarily be assumed to be reliable, economically benefit for farmers and effective alternative methods to prevent and control *Fusarium* contamination. Hence, the objective of this study was to evaluate the antifungal activities of *Terminalia Catappa, Terminalia ivorensis, Terminalia mantaly and Terminalia superba* against *Fusarium* sp. for the development of ecofriendly antifungal compounds for controlling plant diseases caused by *Fusarium* sp., and also to measure their antioxidant potential for application as new natural antioxidant substances.

2. MATERIALS AND METHODS

2.1 Plant Material

The stem barks of *Terminalia cattapa, Terminalia mantaly, Terminalia ivorensis* and *Terminalia superba* were collected at Daloa (Côte d'Ivoire). The identity of each plant material was authenticated at National Floristic Center (NFC) of Felix Houphouet-Boigny University (Cote d'Ivoire, Abidjan) to be identified to specimen NFC 266, NFC 217, NFC 8855 and NFC 10477 respectively for *T. cattapa, T mantaly, T ivorensis* and *T. superba*.

2.2 Microbial Agent

Strains of *Fusarium graminearum, Fusarium oxysporum sp tulipae and Fusarium oxysporum sp radicis-lycopersici* were provided by the Laboratory of Microbiology and Plant Biotechnology, University of Agricultural Sciences and Veterinary Medicine (USAMV) Cluj-Napoca, Romania. These strains were isolated on Potato Dextrose Agar (PDA) from corn seeds, Tulip and tomato infected according to recommendations contained in "the *Fusarium* Laboratory manual" [15].

2.3 Preparation of Plant Extracts

Stem barks of *Terminalia catappa*, *Terminalia mantaly*, *Terminalia ivorensis* and *Terminalia superba* were dried in a dark ventilated room for 5–7 days. These parts were ground to powder and sifted in sieve 0.75 mm size. The extraction of the samples was performed by extraction with SER 148 Velp scientific according to the principle of soxhlet extraction. A mass of 30 g of fine powder of the plant were introduced into 3 cartridges (10 g/cartridge) of the extraction apparatus. Subsequently, these cartridges were introduced separately for 30 minutes into 300 ml of the boiling solvent (Ethanol or Methanol) contained in 3 extraction beakers (100 ml/beaker) according their boiling temperature. After 45 min of refluxing, methanol was completely evaporated and the content of the three beakers were pooled together [16,17]. Then, extract obtained was stored in a refrigerator pending further investigations (antifungal test).

2.4 Evaluation of Antifungal Activity

Antifungal assays were performed on culture medium PDA. Incorporating the plant extract agar was made by agar slant double dilution method. For each of the tests, the series included 9 test tubes with 7 tubes tests containing the plant extracts and 2 control tubes used as growth control and control of sterility of the culture medium. The concentrations of the extracts of these tubes ranged from 1.562 µg/ml to 100 µg/ml binding by a geometrical progression of ½. All 9 tubes were autoclaved at 121°C, 1 bar for 15 minutes and then the tubes were inclined to room temperature so that the agar formed an oblique slope and a 3 cm base. The fungi cultures in media previously prepared was made by inoculation of 1000 cells of the *Fusarium* strains corresponding to 10 µL inoculum 10^{-1} suspension containing 10^5 cells/ml. To prepare the inoculum, a well isolated colony from a six-day culture of the fungal strain was taken with a calibrated loop, transferred in 10 ml of sterile distilled water, homogenised by the vortex and gave a suspension 10° containing 10^6 cells/ml, then 1 ml of this suspension was transferred to 9 mL of sterile distilled water giving a suspension 10^{-1} containing 10^5 Cells/ml. For each test, the load of the inoculum was verified by a series of secondary dilutions and confirmed by a spectrometer. Thereafter, the cultures produced were incubated at 25°C for six days. Minimum Inhibitory Concentration (MIC) was determined by the lowest concentration of extract that inhibited visual growth of fungi in an agar dilution susceptibility testing. In addition, for each test, new sterile PDA medium was used for reseeding content of tubes with concentrations superior or equal to MIC values. Then, they were incubated at 25°C for 6 days in the goal to determine Minimum Fungicidal Concentration (MFC). MFC was determined by the tube with lowest concentration of extract with the growth of one colony. Three replicates for each extract concentration and control against the fungi were used [18].

2.5 Determination of Total antioxidant Activity by DPPH Radical Scavenging Activity

DPPH (2, 2' diphenyl-1-picrylhydrazyl) (80 µM) was dissolved in pure ethanol (98%). The radical stock solution was prepared fresh daily. The mixture was shaken vigorously and allowed to stand at room temperature (25°C) in the dark for 10 min. A volume of 250 µl of sample with 1.75 ml radical solution were added to each microplate well. The decrease in absorbance of the resulting solution was monitored at 515 nm for 30 min. The results were corrected for dilution

and expressed in mM (S)-(-)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, also known as Trolox (T). The extracts (0.1 g sample/1 ml solvent) were used [19].

Inhibition percent (I%) was calculated using the formula:

I% = [(AB –AA)/ AB] X 100 where: AB = Absorbance of blank solution; AA = Absorbance of standard solution (t = 30 min).

3. RESULTS AND DISCUSSION

3.1 Antifungal Activities

The results presented in Table 1 revealed that the methanolic and ethanolic extract of stem barks of *Terminalia catappa, Terminalia ivorensis, Terminalia mantaly and Terminalia superba* notably inhibited *in vitro* growth of *Fusarium oxysporom sp Radicis-lycopersici, Fusarium oxysporum sp tulipae and Fusarium graminearum* at MIC values varying between 3.125 µg/ml and 50 µg/ml. The study revealed that the methanolic extracts generally showed the lowest minimum inhibitory concentration (MIC comprised between 3.125 µg/ml and 25 µg/ml) compared to the ethanolic extracts. In all the study, metahonlic extracts of each *Terminalia* species showed the best activity compared to the ethanolic extracts. In addition, *T. ivorensis* was the most effective plant against these plants pathogens and the most susceptible fungi was *Fusarium oxysporum* sp *tulipae*.

For *Fusarium oxysporum* sp *radicis lycopercisi*, concerning the methanolic extracts, activities of *T. ivorensis* and *T. superba* with MIC and MFC values equal 12.5 µg/ml were better than activities of T. catappa and T. mantaly with values MIC=MFC= 25 µg/ml. Considering the ethanolic extracts, the best activities were observed with T. catappa and T. superba (MFC= 50 µg/ml) whereas the lest activities were obtained with T. ivorensis and T. mantaly (MFC= 100 µg/ml).

For *Fusarium oxysporum* sp tulipae, according to the efficiency order of methanolic extracts, MFC values obtained were 3.125 µg/ml, 6.25 µg/ml, 12.5 µg/ml and 25 µg/ml respectively for T. ivorensis, T. mantaly, T. superba and T. catappa. Concerning the ethanolic extracts, the best activity was observed with T. mantaly (MFC= 25 µg/ml), the lest activity was obtained with T. catappa (MFC = 100 µg/ml), *T. ivorensis* and *T. superba* have shown moderate activity (MFC= 50 µg/ml).

For *Fusarium graminearum*, concerning methanolic extracts, *T. ivorensis* and *T. mantaly* showed equivalent antifungal activities (MIC=MFC=12.5 µg/ml), *T. superba* had a moderate effect (MFC= 25 µg/ml) and *T. catappa* was less effective (MFC= 50 µg/ml). For the ethanolic extracts, *T. ivorensis* and *T. mantaly* (MIC=MFC= 25 µg/ml) have the better effect than *T. catappa* and *T. superba* (MFC= 100 µg/ml).

Similar studies directed by Okigbo and Ogbonnaya [20] confirms a reduced antimicrobial activity with ethanolic extracts against micro-organisms which could be attributed to incomplete solubility of the bioactive compounds in ethanol or the presence of some possible inhibitors. In addition, the variation in activity between the extracts of the same plant may be due to the difference in polarity between the solvents used. The finding of this study is in agreement with the study of previously reported authors, who suggested that same plants may have different antimicrobial activity in different solvents [21].

As the extracts are a complex mixture of secondary metabolites, the observed antifungal activity may be explained in part by possible synergistic interaction between the compounds present in the extract [22]. For all the tests, methanolic extract were fungicidal (MIC and MFC are identical) whereas ethanolic extracts were sometimes fungistatic (MIC and MFC are different). *F graminearum* was the least sensitive fungi with higher MIC and MFC values while *F. oxysporum* sp *tulipae* was the most susceptible. The sensitivity of microorganisms to antimicrobial agents is firstly dependent on the cell wall permeability, the sensitivity of *Fusarium* to the methanolic and ethanolic extracts therefore suggests that the extracts have permeability ability over the cell wall of these *Fusarium species*. This result was similar with the work done by Sharma et al. [23]. The selected *Terminalia* were well known for their antimicrobial properties, but most of the study was carried out with human pathogens [24,25]. This study proved that this genus was also effective against plant pathogens. *Teminalia* species extracts tested may be used for formulating new safer and ecofriendly fungicides.

Table 1. MIC and MFC values of *Terminalia* species extract's

***Fusarium* strains**		***Terminalia catappa***		***Terminalia ivorensis***		***Terminalia mantaly***		***Terminalia superba***	
		Eth	**Meth**	**Eth**	**Met**	**Eth**	**Meth**	**Eth**	**Meth**
F. oxysporum sp radicis-lycopersici	MIC (µg/ml)	25	25	50	12.5	50	25	50	12.5
	MFC (µg/ml)	50	25	100	12.5	100	25	50	12.5
F. oxysporum sp tulipae	MIC (µg/ml)	50	25	25	3.125	12.5	6.25	25	12.5
	MFC (µg/ml)	100	25	50	3.125	25	6.25	50	12.5
F. graminearum	MIC (µg/ml)	50	50	25	12.5	25	12.5	50	25
	MFC (µg/ml)	100	50	25	12.5	25	12.5	100	25

Key: Meth: Methanol; Eth: Ethanol

3.2 Antioxidant Potential

Antioxidant activity was recorded in terms of equivalent factor (F) in Fig. 1 as shown and their equivalent in Inhibition (I). It was observed that these activities were better for methanolic extracts. For these extracts, the highest activity was obtained for *T ivorensis* at 462.34 mM Trolox/1 ml extract (I= 58%), the lowest activity was for *T. superba* with 330.214 mM Trolox/1ml extract (I= 41%), *T. catappa* and *T. mantaly* have medium antioxidant activities at 401.025 mM Trolox/1ml extract (I= 50%) and 385.209 mM Trolox/1 ml extract (48%) respectively. For ethanolic extracts, *T. ivorensis* with 444.414 mM Trolox/1 ml extract (I= 56%) followed by *T. catappa* with 370.205 mM Trolox/1ml extract (I= 46%), *T. mantaly* with 345.135 mM Trolox/1ml extract (I= 43%) and *T. superba* with 295.147 mM Trolox/1 ml extract (I= 37%). In comparison to *Cymbopogon citratus* extracts evaluated in the same conditions, all these antioxidant activities were better [17]. Similar DPPH scavenging activities of different plant extracts had been observed by different researchers [26,27]. As *Terminalia* species produce significant amount of antioxidants, they could be considered as potential source of phenolic compounds such as acids phenolic and flavonoids, which their dietary intake can help us to prevent the oxidative stress caused by photons and oxygen. In addition, the presence of these compounds in tested extracts of *Terminalia* species could explain their antifungal activity against *Fusarium*. The use of

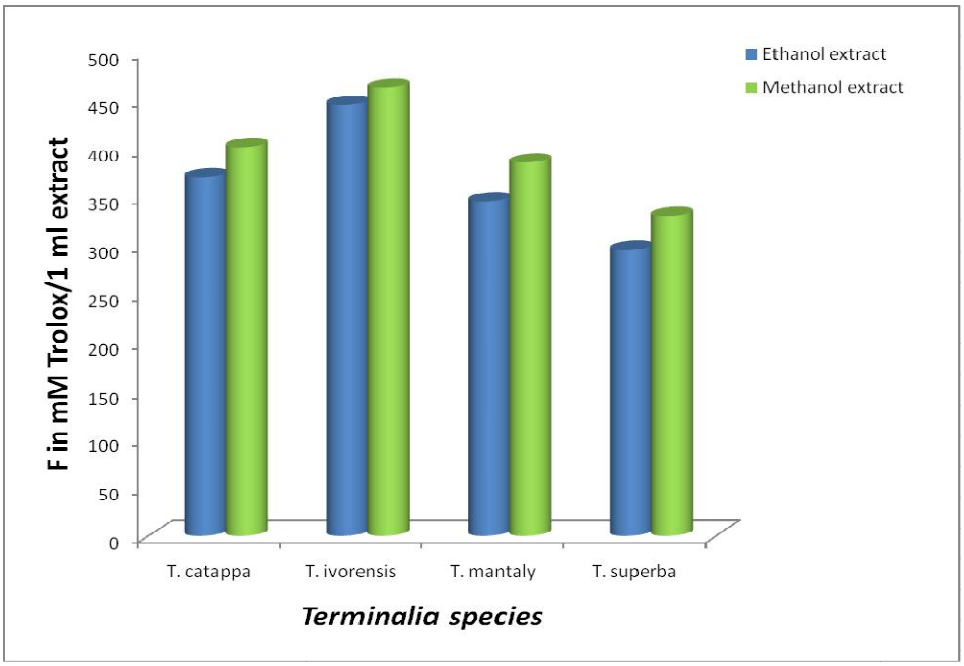

Fig. 1. Histograms of antioxidant activities of *Terminalia* species

synthetic antioxidants (BHA, BHT, TBHQ) has limitations such as toxicological risk, development of cancer and hence natural antioxidants have gained importance. The excellent antioxidant activities of *Terminalia* species demonstrated that these barks stem of *Terminalia* presented high concentrations in total phenolics compounds.

This study showed that *Terminalia* species possessed interesting antioxidant and antifungal activities.

4. CONCLUSION

The barks stem extracts of *Terminalia Catappa*, *Terminalia ivorensis*, *Terminalia mantaly* and *Terminalia superba* possessed both antifungal activities against *Fusarium* species and antioxidant potency. These plants could be used on the on hand to develop new biofungicides for controlling *Fusarium* contamination without harm effects on Human, and the other hand for the discovery of natural antioxidant substances for preventing some diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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