

Original Research Article

IN VITRO ANTIOXIDANT POTENCY AND ANTIFUNGAL EFFICIENCY OF FOUR LOCAL *TERMINALIA* SPECIES AGAINST *FUSARIUM* STRAINS

ABSTRACT (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

ABSTRACT:

Aims: To evaluate *in vitro* antifungal activities of four selected *Terminalia* species and their antioxidant potencies.

Study design: Activities directed antifungal and antioxidant potency of extract's from *Terminalia* species *in vitro* methods.

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.

Methodology: Growth inhibitory effect of ethanolic and methanolic extracts of *Terminalia* species against *Fusarium oxysporum* sp *tulipae*, *Fusarium oxysporum* sp *radicis-lycopersici* and *Fusarium graminearum* were tested *in vitro* by applying agar slant double dilution method and the measurement of antioxidant activity of the tested extracts was done through DPPH scavenging method.

Results: All test fungi were susceptible to both extracts of *Terminalia* sp extracts in dose-effect relationship. Methanolic extracts of each *Terminalia* sp. tested had lowest parameters 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 cause "Fusariosis" in more than a hundred species of plants such as tomato, maize, sugarcane, Tulip, *Musa* spp., etc. They colonize 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;

Comment [RVW1]: Recast, the sentence is unclear

Comment [RVW2]: Why is this italicized?

Comment [RVW3]: Whichpart of the plant was used?

Comment [RVW4]: The methodology is not detailed. Name the plant species too

Comment [RVW5]: Of all the species? Please specify

Comment [RVW6]: Parameters??? You mean the values??

Comment [RVW7]: Use common name for uniformity

25 3]. Thus, these plant extract could be also a source of natural antioxidant substances which can allow
 26 to fight against oxidative stress [4; 5].
 27 *Terminalia* species belonging to the family *Cecombretaceae*, are rich in polyphenolic components and
 28 triterpenoids [6; 7] and could be species for the development of new natural biofungicides. Different
 29 parts of the plants of this genus have long been used as folk medicine in western Africa for
 30 antidiarrheic, antipyretic and hemostatic purposes [8; 9]. In several countries, researchers have used
 31 the leaves, bark, and fruit of some species of *Terminalia* to treat dermatitis and pyresis [10].
 32 Moreover, the anti-inflammatory ~~activity~~, hepatoprotective ~~property~~, antidiabetic, carcinogenesis-
 33 preventing ~~effects~~, antimalarial~~ic~~, antinociceptive and antimicrobial [11; 12; 13] activities of *Terminalia*
 34 species have been studied and this genus could potentially provide benefits to humanity in agricultural
 35 domain and human healthcare. In Côte d'Ivoire, ~~it~~ has been reported by earlier researchers that
 36 *Terminalia catappa* L., *Terminalia ivorensis* A Chev, *Terminalia mantaly* H. Perrier and *Terminalia*
 37 *superba* (Engl. & Diels) were found to possess good antifungal activities against human pathogen~~sic~~ by
 38 reducing the *in vitro* growth of some yeast and moulds [14]. ~~To our knowledge, so far no investigation~~
 39 ~~has been reported regarding the activities of the stem bark of local Terminalia species against~~
 40 ~~Fusarium contamination~~. Hence, the objective of this study ~~is was~~ to evaluate the antifungal activities of
 41 these four local *Terminalia* species ~~(list)~~ against *Fusarium* sp. for the development of ecofriendly
 antifungal
 compounds for controlling plant diseases caused by *Fusarium* sp, and also to measure their
 4342 antioxidant potential for application ~~as for~~ new natural antioxidant substances.

2. MATERIAL AND METHODS

2.1 Plant material

The stem bark 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 Houphouët-Boigny University (Cote d'Ivoire, Abidjan) to be
 identified. ~~These plants are used in traditional environment to treat various fungal diseases.~~

2.2 Microbial agent

Strains of *Fusarium graminearum*, *Fusarium oxysporum* sp *tulipae* and *Fusarium oxysporum* sp
radicis-lycopersici ~~plant pathogenic fungi~~ were provided by the Laboratory of Microbiology and ~~P~~plant
 Biotechnology, University of Agricultural Sciences and Veterinary Medicine (USAMV). Cluj-Napoca,
 Country?.

These strains were isolated on ~~PDA~~ (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 bark~~s~~ of *Terminalia catappa*, *Terminalia mantaly*, *Terminalia ivorensis* and *Terminalia superba*
~~plant material~~ 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 sample was performed ~~by extraction with SER~~
 148 Velp scientific according to soxhlet extraction. A mass of 3 x 10 g of fine powder of the plant were
 introduced into 3 cartridges of the extraction apparatus. Subsequently, these cartridges were
 introduced separately for 30 minutes into 3 x 100 ml of the boiling solvent (Ethanol or Methanol)

Comment [RVW8]: What does earlier researchers mean in this context? Use

Comment [RVW9]: You may want to review this statement. Remove, preferably.

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Comment [RVW10]: Include deposit voucher number

Comment [RVW11]: This information should be stated and explicitly written in the "introduction" section

Comment [RVW12]: This is unclear

Comment [RVW13]: Write out the cumulative weight

Comment [RVW14]: Cumulative volume...e.g 300 ml

66 contained in 3 extraction beakers according their boiling temperature. After 45 min of refluxing,
67 methanol was completely evaporated and the content of the three beakers were pooled together [16;
68 17].Then, extract obtained was stored in a refrigerator pending further investigations (antifungal test).

70 2.4 Evaluation of antifungal activity

71 Antifungal assays were performed on culture medium, PDA (Potato Dextrose Agar). Incorporating the
72 plant extract agar was made by the method of double dilution in inclined tubes. The Our series includes
73 test tubes with 07 tubes tests (containing the plant extracts) and 2 control tubes (without a plant extract
74 used as growth control of the organisms and the other fungi-free and without extract served as a and
75 control of sterility of the culture medium). The concentrations of these tubes ranged from 100 to 1.562
76 µg/ml binding by a geometrical reason of ½. All 9 tubes were autoclaved at 121 °C, 1 bar for 30
77 minutes and then the tubes were inclined to room temperature
77 of the laboratory to cooling and

78 solidification of agar. The fungi cultures in media previously prepared was made by inoculation of
79 1000 cells of the *Fusarium* strains corresponding to 10 µL inoculum 10^{-1} suspension containing 10^5
80 cells/ml. This inoculum was obtained by sampling with a loop diameter of 2 mm with a sterile single
81 colony which was homogenized in 10 ml of distilled water giving a suspension containing 10^6 cells/ml.
82 then 1 ml of this suspension was transferred to 9 mL of sterile distilled water giving a suspension 10^{-1} .
83 For each test, the load of the inoculum was verified by a series of secondary dilutions (ref?). Thereafter, the
84 cultures produced were incubated at 25°C for six days of incubation. Minimum Inhibitory
85 Concentration (MIC) was determined by the lowest concentration of extract that inhibited visual
86 growth of fungi in an agar dilution susceptibility testing. In addition, for each test, new sterile PDA
87 medium was used for reseeding content of tubes with concentrations superior or equal to MIC values.
88 Then, they were incubated at 25°C for 6 days in the goal to determine Minimum Fungicidal
89 Concentration (MFC). MFC was determined by the tube with lowest concentration of extract with the
90 growth of one colony. Three replicates for each extract concentration and control against the fungi
91 were used [18]. Was there the use of any standard antifungal for comparison??

92 2.5 Determination of Total antioxidant Activity by DPPH Radical Scavenging Activity

93 DPPH (2, 2' diphenyl-1-picrylhydrazyl) (80 µM) was dissolved in pure ethanol (98%). The radical stock
94 solution was prepared fresh daily. The mixture was shaken vigorously and allowed to stand at room
95 temperature (25°C) in the dark for 10 min. A volume of 250 µl of sample with 1.75 ml radical solution
96 were added to each microplate well. The decrease in absorbance of the resulting solution was
97 monitored at 515 nm for 30 min. The results were corrected for dilution and expressed in mM (S)-(-)-
98 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, also known as Trolox (T). The extracts (0.1 g
99 sample/1 ml solvent) were used [19].

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

101 $I\% = \left[\frac{(AB - AA)}{AB} \right] \times 100$ where: AB = Absorbance of blank solution; AA = Absorbance of standard
102 solution (t = 30 min).

104 3. RESULTS AND DISCUSSION

105 3. 1 Antifungal activities

Comment [RVW15]: Are you sure this is what the method described here is called? And the use of "inclined tubes" for slants is quite ridiculous. Support with a valid reference

Comment [RVW16]: The use of the pronoun is unacceptable!

Comment [RVW17]: Was this setup for both ethanol and methanol extracts? Then for all the organisms???

Comment [RVW18]: The concentration of the tubes or the content? How did you vary concentration? How was the extract reconstituted for bioassay?

Comment [RVW19]: Range is written as lower to higher regardless if it is reverse dilution

Comment [RVW20]: You mean progression? Please check word

Comment [RVW21]: With the contents aforementioned?? Was the medium sterilized with the plant extracts in it?

Comment [RVW22]: Is there a specific reason for increasing sterilization time??

Comment [RVW23]: Inclined? How?

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Comment [RVW24]: This is confusing! How did you prepare the fungal suspension?

Comment [RVW25]: What was sampled with a loop??

Comment [RVW26]: How can a microbial colony be sterile???? This is ridiculous

Comment [RVW27]: What was used for the hyphal homogenization?

Comment [RVW28]: Are you claiming that the cells were diluted from 10^6 cells/ml to 10^{-1} with one transfer??? Impossible! What was the final fungal cell load used for the assay?

Comment [RVW29]: How was the load confirmed? Was there any confirmation with a spectrometer??

106 The results presented in Table I revealed that stem bark extract's of all four *Terminalia* species
 107 significantly inhibited *in vitro* growth of *Fusarium oxysporum* sp *Radicalis-lycopersici*, *Fusarium*
 108 *oxysporum* sp *tulipae* and *Fusarium graminearum* at MIC values varying between 50 and 3.125
 109 µg/ml. Methanolic extracts of *Terminalia* species caused highest inhibition of fungi growth (MIC
 110 comprised between 3.125 µg/ml and 25 µg/ml). [The study revealed that the methanolic extracts
 111 generally showed the lowest minimum inhibitory concentration compared to the ethanolic extracts.
 112 Considering activities of all the extracts of *Terminalia*, *Terminalia ivorensis* extracts showed the best
 113 activities. For the comparison between activities of plant extracts, When, the extracts showed the
 114 same MIC values, we consider the MFC values to make the comparison. The lowest values (MIC or
 115 MFC) mean that the extract was efficient .
 116 On-For *Fusarium oxysporum* sp *Radicalis lycopersici*, the highest activities were observed with the
 116 considering the methanolic extracts the highest
 117 inhibition was obtained both by *T. ivorensis* and *T. superba* (MIC=MFC= 12.5 µg/ml), followed by *T.*
 118 *catappa* and *T. mantaly* (MIC=MFC= 25 µg/ml) whereas for the ethanolic extracts the effectiveness
 119 was for *T. catappa* (MIC= 25 µg/ml) followed by *T. ivorensis* and *T. superba* (MFC=50 µg/ml) and *T.*
 120 *mantaly* (MFC= 100 µg/ml).
 121 On *Fusarium oxysporum* sp *tulipae* , according to the efficiency of methanolic extracts, we have *T*
 122 *ivorensis* (MIC=MFC= 3.125 µg/ml), *T. mantaly* (MIC= MFC= 6.25 µg/ml), *T. superba* (MIC= MFC=
 123 12.5 µg/ml) and *T. catappa* (MIC=MFC= 25 µg/ml) while for the ethanolic extract, the best activities
 124 were obtained in the following order: *T. ivorensis* (MIC= 12.5 µg/ml), *T. mantaly* (MIC= 25 µg/ml), *T.*
 125 *superba* (MIC= 25 µg/ml) and *T. catappa* (MIC= 50 µg/ml).
 126 On *Fusarium graminearum*, for the methanolic extracts, *T. ivorensis* and *T. mantaly* have the same
 127 efficiency (MIC= 12.5 µg/ml) followed by *T. superba* (MIC= 25 µg/ml) and *T. catappa* (MIC= 50 µg/ml)
 128 while for the ethanolic extracts *T. ivorensis* and *T. mantaly* have identical MIC and MFC values (25
 129 µg/ml) and *T. catappa* and *T. superba* are also the same efficiency (MIC= 50 µg/ml; MFC= 100
 130 µg/ml). The ethanolic extracts showed less antimicrobial activity compared to the methanolic extract.
 131 Similar studies directed by [20] confirms a reduced antimicrobial activity with ethanolic extracts
 132 against microorganisms which could be attributed to incomplete solubility of the bioactive compounds
 133 in ethanol or the presence of some possible inhibitors. In addition, the variation in activity between the
 134 extracts of the same plant is-maybe due to the difference in polarity between the solvents used. The
 135 finding
 136 of this study is in agreement with the study of previously reported authors, who suggested that same
 136 plants may have different antimicrobial activity in different solvents [21].
 136 The possible reason for this
 137 effect is that each plant contains different components in the form of secondary metabolites that have
 138 different characteristic effect in various solvents which varies on the basis of physical and chemical
 139 properties. As the extracts are a complex mixture of secondary metabolites, the observed antifungal
 140 activity may be explained in part by possible synergistic interaction between the compounds present
 141 in the extract [22]. For all the tests, methanolic extracts were fungicidal (MIC=MFC) whereas
 142 ethanolic extracts were sometimes fungistatic (MIC and MFC are different). *F. graminearum* was the
 143 least sensitive fungi with higher MIC and MFC values while *F. oxysporum* sp *tulipae* was the most
 144 susceptible. The sensitivity of microorganisms to antimicrobial agents is dependent on the cell wall

Comment [RVW30]: The use of this word should be avoided if no statistical comparison was done. Synonyms like "notably" should be considered.

Comment [RVW31]: Refer to comment on writing range

Comment [RVW32]: Wrong grammatical construction. Rewrite!

Comment [RVW33]: This is very UNCLEAR and POORLY WRITTEN. Check grammar!

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Comment [RVW35]: Rewrite!

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Comment [RVW36]: Repetition!

Comment [RVW37]: What does this mean?

145143 permeability of antifungal agents. The sensitivity of *Fusarium* to the methanolic and ethanolic extracts

Comment [RVW38]: Is this the sole reason??

therefore suggests that the extracts have permeability ability over the cell wall of these fungi which serve as a barrier to most commonly used antimicrobial agents. This result was similar with the work done by some authors [23] who aimed to the presence of antimicrobial substances in the higher plants is well established. 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. *Terminalia* species extracts tested may be used for formulating new safer and ecofriendly fungicides.

Table I: MIC and MFC values of *Terminalia* species extract's

<i>Fusarium</i> strains		<i>Terminalia catappa</i>		<i>Terminalia ivorensis</i>		<i>Terminalia mantaly</i>		<i>Terminalia superba</i>	
		Eth	Meth	Eth	Met	Eth	Meth	Eth	Meth
<i>F. oxysporum sp radialis-lycopersici</i>	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
<i>F. oxysporum sp tulipae</i>	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
<i>F. graminearum</i>	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 Figure 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/1ml 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/1ml extract (48%) respectively. About for ethanolic extracts, we have *T. ivorensis* with 444.414 mM Trolox/1ml 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/1ml 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 antioxidant compounds which their dietary intake can help us to prevent the oxidative stress caused by photons and oxygen. The use of synthetic antioxidants has limitations and hence natural antioxidants have gained importance. The excellent antioxidant activities of *Terminalia*

Comment [RVW39]: REALLY? Which ones??

Comment [RVW40]: There is consistent use of "some authors" with just one reference cited to support the claim

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Comment [RVW41]: Check grammar

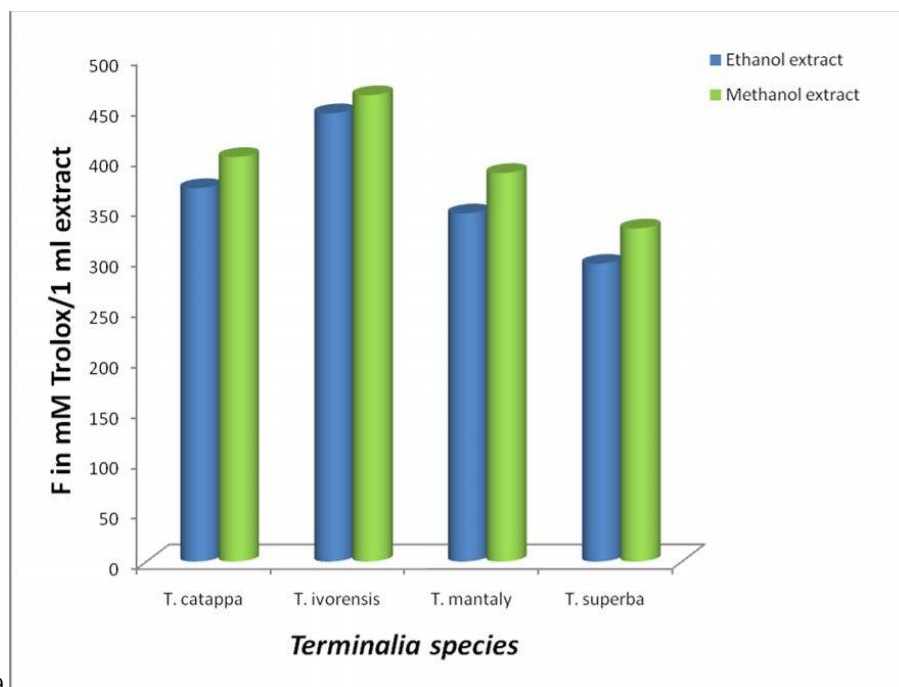
Comment [RVW42]: Why the comparison to *Cymbopogon*??

Comment [RVW43]: What is the significance of the antioxidant property in relation to its biological activity on *Fusarium*??

Comment [RVW44]: Such as?

175 species demonstrated that these barks stem of *Terminalia* were richness in total phenolics and could
 176 also explain the antifungal activities observed.
 177 This study showed that *Terminalia* species possessed interesting antioxidant and antifungal activities.

Comment [RVW45]: Check grammar



179
 180
 181 **Figure 1:** Histograms of antioxidant activities of *Terminalia* species

183 4. CONCLUSION

184 The barks stem extracts of *Terminalia Catappa*, *Terminalia ivorensis*, *Terminalia mantaly* and
 185 *Terminalia superba* possessed both antifungal activities against *Fusarium* species and antioxidant
 186 potency. This is an essential basis for the development of new biofungicides for controlling *Fusarium*
 187 contamination without harm effects on Human and for the discovery of natural antioxidant substances
 188 for preventing some diseases. Among *Terminalia* species tested, *Terminalia ivorensis* extracts
 189 showed both the best antifungal activities with MIC varying between 3.125 and 50 µg/ml and the
 190 highest antioxidant potential with values at 462.34 mM Trolox/1 ml extract and 444.4114 mM Trolox/1
 191 ml extract. The most susceptible fungi was *Fusarium oxysporum* sp *tulipae*.

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Comment [RVW46]: Conclusion is not an avenue for repetition of results. Summarize the notable observations. Rewrite!

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