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Antifungal Activity of the Essential Oil of Elionurus Muticus (Spreng) Kunth from Zimbabwe against Candida albicans, C. krusei and Cryptococcus neoformans

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***Abstract*—Cultivated Elionurus muticus (Spreng.) Kunth (Gramineae) plant parts were steam distilled for essential oil and evaluated in-vitro for antifungal activity against Candida albicans, C. krusei and Cryptococcus neoformans using agar broth dilution and disc diffusion methods and the activity compared with commercial citral and Cymbopogon citratus DC (Stapf) (lemongrass) oils by measuring the growth inhibition diameter. E. muticus oils showed fungicidal activity with MIC and MFC values between 0.5-10µg/mL. E. muticus whole plant oil showed greater antifungal activity against C. albicans than citral and lemongrass oils. E. muticus whole plant essential oil contains citral and $\Delta^{1(10)}$aristolen-2-one as major components, both are known antifungal compounds. Their unique presence in one plant extract could have significant impact in treating mycotic diseases and other opportunistic infections and for use in clinical aromatherapy and in complementary and alternative medicines.**

Keywords—Elionurus muticus (Spreng) Kunth; essential oil; antifungal activity; Candida spp.; Cryptococcus neoformans

I. INTRODUCTION

Treatment of HIV/AIDS and some non-communicable diseases (NCDs) is becoming increasingly difficult due to microbial resistance and opportunistic infections due to *Candida* and *Cryptococcus* spp., methicillin resistant *Staphylococcus aureus* and *Herpes Simplex* Virus I and II.(HSV.I/II) [1-7]. Invasive fungal infections are considered difficult to manage; however, the use of essential oils including citral based oils is offering increased therapeutic outcomes and strategies [8]. The physicochemical properties of essential oils are perceived advantageous to increasing cell permeability and eventually cell death [7].

Citral and some citral based oils: lemongrass (*Cymbopogon citratus*), *Lippia alba* (Mill), *Elyonurus muticus*, *Elionurus hensii* (Schum), *Melissa officinalis* and *Litsea cubeba*, have been studied for their antifungal, cytotoxicity and other biological activities [1,9-15]. *Elionurus muticus* (Spreng) is a tufted grass found in Zimbabwe and has been evaluated for essential oil composition, antioxidant activity and nutritional composition. The aerial parts of the essential oil contains citral (60-80%) whilst the root contains $\Delta^{1(10)}$aristolen-2-one (~70%) as major component (Fig.1) [16,17,18]. Both bioactive compounds have been reported for their antifungal activity apart from the other well-known uses for citral in the flavour, chemical and fragrance industries [12-19]

We now report a study carried out to assess the antifungal activity through the growth inhibition of *Candida albicans*, *C. krusei* and *Cryptococcus neoformans* by *E. muticus* whole, aerial and root plant essential oils and to compare growth inhibition with commercial citral and *Cymbopogon citratus* (DC) Stapf. (lemon grass) oils [20].

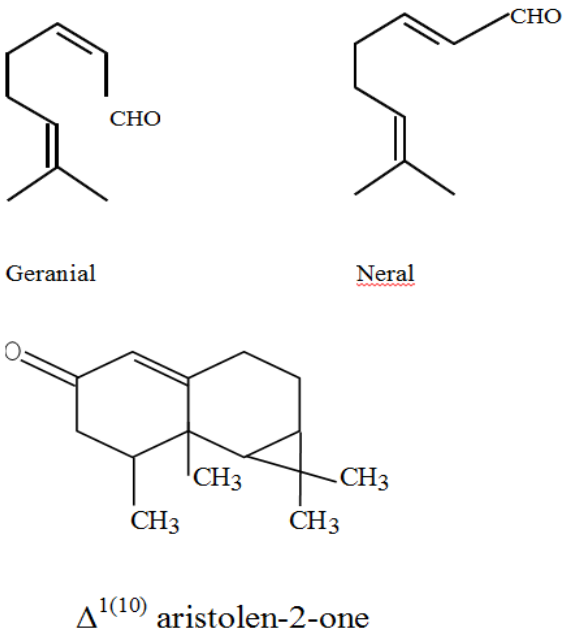

Figure 1: The chemical structures of geranial, neral and $\Delta^{1(10)}$ aristolen-2-one

II. MATERIALS AND METHODS

A. Plant essential oil and citral

The steam distilled essential oils from *E. muticus* (Spreng) Kunth and *C. citratus* (DC) Stapf (lemon grass) plant parts and commercial citral were obtained from own stocks previously analysed and reported [16,17,21].

B. Antifungal Screening

The agar broth dilution (MIC and MFC) and disc diffusion (Inhibition Zone) methods were used for antifungal screening [22,23]. *Candida albicans* (ATCC90021) *C. Krusei* (ATCC6258) and *Cryptococcus neoformans* (ATCC30951) fungal strains were obtained from the mycology laboratory, Department of Medical Microbiology, University of Zimbabwe. They were cultured and subcultured on Sabaraud dextrose agar (SDA) slates and fungal inoculum adjusted to $1X10^5$ CFU/mL before use.

C. Broth Dilution Assay

Predetermined weights (200-4000µg) of the essential oils were emulsified with 10% Tween 80 (in water) and aqueous Sabaraud dextrose broth (SDB) added gradually until 3.6mL and eventually made up to 4mL with the fungal culture. Three controls were set; one containing 0.4mL of the fungal strain and 0.02% Tween 80 in SDB, another containing SDB and the fungal strain and the third containing only SDB. The tubes were incubated at 36^0C for 24 hours for the *Candida* spp. and 48 hours for *Cryptococcus neoformans.* The suspension in each test tube was serially diluted (10- fold) with SDB to a final concentration of 10^{-4} and 0.4mL of the 10^{-3} and 10^{-4} were plated out onto potato dextrose agar and counted.

D. Disk Diffusion Assay

An overnight culture of fungi (0.5 mL) was spread over the surface of 20mL agar plates and incubated for 30 minutes. 5µg of each of the essential oils was placed on 6mm blank antimicrobial susceptibility discs. The oil impregnated discs were then placed onto the inoculated surface of the agar plate (maximum of five disks per plate and each impregnated with a different essential oil). The agar plates were incubated overnight at 37°C and the growth inhibition zone diameters (IZD) were recorded using a 15cm ruler. Two determinations were carried out; the first (IDZ_1) using essential oils from *E. muticus* alone and the second (IDZ_2) using all the 5 essential oils mentioned above. The plates were prepared in triplicate.

E. *Statistics*

Data was entered into a computer and analyzed using GraphPad® software. IZD values were expressed as mean ± S.D. Two-way analysis of variance (ANOVA) followed by Benferroni posttests was used at 95% Confidence Interval (α=0.05).

III. RESULTS AND DISCUSSION

Broth dilution assays showed that *E. muticus* essential oils had MIC values ranging that from 0.5-5µg/mL and MFC values of 2.5-10µg/ml against the *Candida* albicans, *C. krusei* and *Cryptococcus neoformans* (Table 1). *C. albicans* was the most susceptible to whole and aerial essential oils with MIC values of 0.5µg/mL. *C. krusei* was more susceptible to aerial than root essential oil whilst *C.neoformans* was more susceptible to aerial than whole plant essential oil with MIC and MFC values of 1.0 and 10µg/mL respectively.

Disc diffusion assays showed that *E.muticus* whole plant essential oil (IDZ_2=41.7±1.5mm), lemongrass oil (IDZ_2=26.3±1.5mm) and citral (IDZ_2=27.3±2.5mm) inhibited the greatest fungal growth against *C. albicans* and less so against *C. krusei* (IDZ_2=18.7±4.2-20.7±1.5mm) and *C. neoformans* (IDZ_2=12.0±1.0-19.0±1.0mm) (Table 2, Fig.2). The results also showed that *C. albicans* was the most susceptible strain while *Crypt. neoformans* was the least susceptible to the essential oils tested. *E. muticus* whole essential oil was the most active against *C. albicans* (IDZ_1=46±2.8mm, IDZ_2=41.7±1.5mm) whilst its aerial part essential oil was comparable in activity with the citral and lemongrass oils against *C. krusei* and *C.neoformans*.

Plant part	% Major constituents		*Candida albicans*		*C. krusei*		*Crypt. neoformans*	
	Citral	$\Delta^{1(10)}$ aristolen-2-one	MIC µg/mL	MFC µg/mL	MIC µg/mL	MFC µg/mL	MIC µg/mL	MFC µg/mL
Root	1	70	1.0	5.0	5.0	10.0	2.5	5.0
Whole Plant	30	55	0.5	2.5	2.5	5.0	1.0	2.5
Aerial Parts	72	-	0.5	2.5	1.0	5.0	5.0	10.0

Table 1:The MIC and MFC values (ug/mL per disc) for *E. muticus* essential oils against *Candida albicans*, *C. krusei* and *Cryptococcus neoformans*

Plant part essential Oil (% citral)	Diameter of Inhibition Zone (IDZ)(mm) n=3					
	Candida albicans		*C. krusei*		*Crypt. neoformans*	
	IDZ_1	IDZ_2	IDZ_1	IDZ_2	IDZ_1	IDZ_2
E. muticus root (1)	22±2.8	20.0±2.6	13.5±2.1	15.3±2.1	15±1.4	14.3±1.5
Aerial parts (72)	22±2.8	21.0±2.6	20.5±3.5	20.3±2.5	16±1.4	16.7±1.5
Whole plant (30)	46±2.8	41.7±1.5	18.0±2.3	19.0±2.0	10±1.4	12.0±1.0
Citral commercial (95)	-	27.3±2.5	-	20.7±1.5	-	18.7±1.5
Cymbopogon citratus (75)	-	26.3±1.5	-	18.7±4.2	-	19.0±1.0

Table 2: The growth inhibition diameter zones (IDZ) for E. muticus oils (5ug/mL per disc) against *Candida albicans*, *C. krusei* and *Crypt. neoformans* by disc diffusion compared to commercial citral and *C. citratus* grown in Zimbabwe

The biological activity of an essential oil usually correlates with that of its major component(s) [3,13,19,23,24]. Citral, lemongrass oils and their citral-chemotypes have been investigated for antifungal activities against *Candida spp* and other dermatophytes and for their cytotoxicities against Vero non-tumour and HeLa tumour cell lines [11,13]. Citral-chemotypes from Colombian *Lippia alba* (Mill) N.E. Brown (54.1% citral) was fungicidal against *C. krusei* at MIC concentration of 270.8µg/mL compared to commercial citral (99%) with 39.72ug/ml and Amphotericin B positive control, 0.5ug/ml [11].

Citral was cytotoxic to Vero non-tumour cell lines (CC_{50} < 124.1±12.2µg/mL) and (CC_{50} < 0.1µg/mL) against HeLa tumour cell lines. The essential oil of Italian *Cymbopogon flexuosus* with citral (60%) had an MIC of 500ppm (≡499.4µg/mL. Thai *Cymbopogon citratus* containing citral (78%) was fungicidal against selected dermatophytes at concentrations ranging from 115µg/mL to 310µg/mL. Citral was shown to be the compound responsible for the observed biological effects [3].

The fungicidal activity of the essential oils from *E. muticus* from Zimbabwe can be attributed to the presence of citral (25-80%) and $\Delta^{1(10)}$aristolen-2-one (50-72%) whilst that of the lemongrass oil can be attributed to the presence of citral (70-75%) and myrcene (10-19%) [16,17,21]. With MIC value 0.5-2.5µg/ml against *Candida spp., E. muticus* oils can be considered strong inhibitors. *E. muticus* could have major impact on the treatment of *Candida* infections due to the observed additive and/or synergistic effects of its root and aerial major components which are not reflected on its effect on *C. kruesi* and *C. neoformans* as other minor monoterpene and sesquiterpene components: myrcene, geraniol, geranyl acetate, farnesols, other ketones and alcohols, could also enhance or diminish these responses by influencing interactive mechanisms, cell permeability and the lipophilic characteristics of the extracts [1,13,17]. Tests are in progress to evaluate further in-vitro and in-vivo antimycotic responses against standard antimicrobials and clinical isolates and to assess herb-herb, herb-drug and herb-substrate interactions.

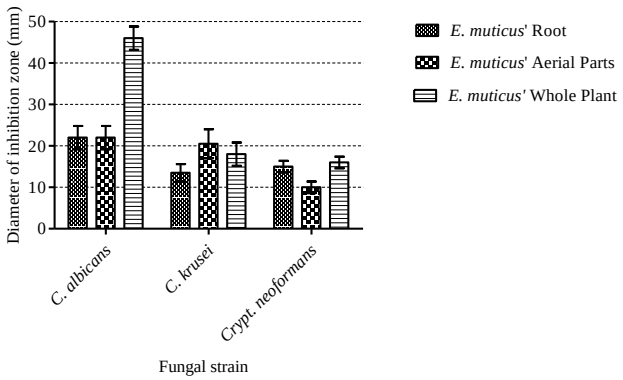

Figure 2. The growth inhibition zone diameters (mm) of the essential oils for *Elionurus muticus* plant parts (5ug/ml) against fungal strains by disc diffusion method.

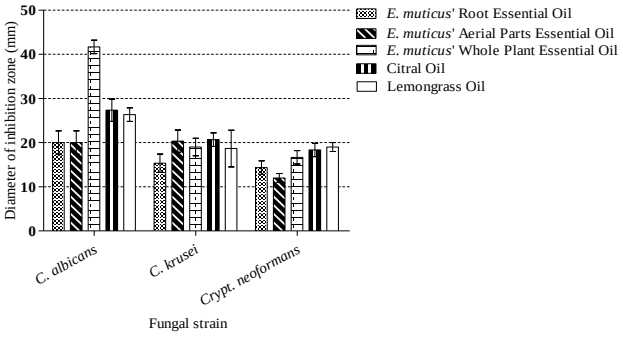

Figure 3. The growth inhibition zone diameters for standard citral, *Cymbopogon citratus* and *Elionurus muticus'* plant part essential oils (5µg per disk) against *Candida albicans, C. krusei* and *Crypt. neoformans* as determined by the Disk Diffusion Assay

IV. CONCLUSIONS

$\Delta^{1(10)}$aristolen-2-one and citral are the major phytochemicals responsible for the observed fungicidal activity of essential oils from *E. muticus*' plant parts. These components could exhibit a synergistic or additive effect on *C. albicans* and related species and overcome their virulence [25]. The essential oils from the roots, aerial parts and whole

plant of Zimbabwean *E. muticus* (Spreng) Kuntze were fungicidal against *C. albicans*, *C. krusei* and *C. neoformans* with the plant essential oil having the most effect and thus have a greater potential for use in the management of HIV/AIDS fungal opportunistic infections.

V. RECOMMENDATIONS

Researchers must consider variations in constituents from different plant parts as well as potential regional differences in compositions for any medicinal plant for a more comprehensive characterization of their biological effects. For studies on plant extracts, the challenge is to identify stable chemotypes and propagate them through pilot cultivations to potential commercial viability. This should also be accompanied by capacity to produce quality extracts, analyse, identify major constituents and to quality control the extracts.

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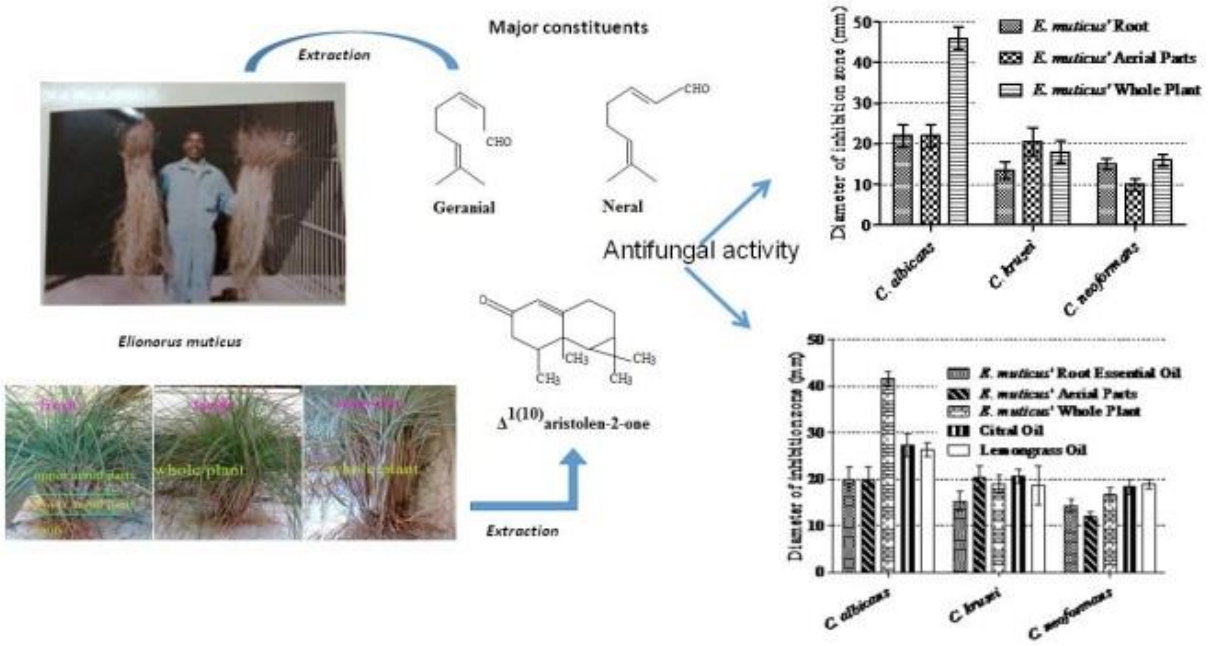

Figure 4. Graphic abstract-Antifungal activity of the essential oil of *Elionurus muticus* (Spreng) Kunth from Zimbabwe against *Candida albicans, C. krusei* and *Cryptococcus neoformans*

Blessed Fungirayi[1] and Lameck Shoriwa Chagonda[1*]