



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(3): 01-05
Received: 01-03-2017
Accepted: 02-04-2017

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In vitro antifungal activity of essential oils extracted from some plants of *Tangawisi*® products on *Candida albicans*

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Abstract

This work aimed to evaluate the antifungal activity of essential oils extracted from *Tangawisi*® plants on a strain of *Candida albicans*. Nine plants were identified as aromatic plants contained in *Tangawisi*® products and their essential oil yield had being determined. The extraction yield in essential oils of identified plants were respectively *Piper guineense* (4.94%), *Monodora myristica* (1.57%), *Zingiber officinalis* (1.31%), *Xylopi aethiopica* (1.23%), *Securidaca longepedunculata* (1.07%), *Cyperus articulatus* (0.59%), *Aframomum melegueta* (0.33%), *Mangifera indica* (0.16%) and *Heinsia crinita* (0.247%). TLC chromatographic profile showed that each essential had a characteristic fingerprint and compared to thymol and eugenol used as positive control, only *Zingiber officinalis* contains eugenol. The diameter of the inhibition zone formed as a result of six of these essential oils is 6 mm for essential oils of *Monodora myristica* and *Piper guineense*, 9.33 mm for that of *Cyperus articulatus*, 11.33 mm for the *Xylopi aethiopica*, 15.7 mm for that of *Securidaca longepedunculata* and 19 mm for the *Zingiber officinalis* oils. The minimum fungistatic concentration on the strain of *Candida albicans* is 0.05% for essential oils of *Securidaca longepedunculata*, 0.075% for *Zingiber officinalis* and *Xylopi aethiopica* and 0.5% for those of *Monodora myristica*, *Piper guineense* and *Cyperus articulatus*. The essential minimum fungicidal concentration is 0.075% for oil of *Securidaca longepedunculata*, 0.25% for that of *Zingiber officinalis*, 0.5% for that of *Xylopi aethiopica*, 1% for that of *Monodora myristica*, 2.5% for that of *Cyperus articulatus* and is greater than 3.5% for that of *Piper guineense*. The essential oils of *Securidaca longepedunculata*, *Zingiber officinalis* and *Monodora myristica* have a fungicide power to the *Candida albicans* strain while those of *Xylopi aethiopica*, *Cyperus articulatus* and *Piper guineense* have a fungistatic one.

Keywords: Tangawisi, essential oil, TLC chromatographic profile, *Candida albicans*, antifungal activity

Introduction

Candida albicans is the most frequent aetiological agent for candidiasis and is capable of causing any clinical mycoses. It is usually a superficial but troublesome infection that may occur in intertriginous areas, nails with adjacent tissues, mucosae of the mouth, pharynx, and vagina. Under certain conditions, *C. albicans* may also infect the bronchopulmonary system, kidney, and heart. *C. albicans* is also implicated in congenital cutaneous candidosis usually associated with various skin manifestations [1].

The widespread overuse of broad-spectrum antibiotics also appears to have encouraged a new problem. It is often the cause of secondary infection by yeasts, especially *Candida albicans* [2]. Modern antifungal drugs have limited efficacy and cause considerable adverse effects such as gastrointestinal disturbances, cutaneous reactions, hepatotoxicity and leucopenia in some of the treated patients [3].

Plants and their preparations have been used as remedies against infectious diseases. Inhibition of growth to several fungi has been verified in our previous studies [4]. Plant-derived compounds comprise safer and more effective substitutes for synthetically produced antimicrobial agents. Essential oils are one of the most promising groups of natural compounds attracting scientific interest because of their antifungal activity against yeast and filamentous fungi [3]. Essential oils have demonstrated clinical efficacy for the treatment of otitis media, dermatophyte infections and oral and vaginal candidiasis in animals in addition to oral and vaginal infections, acne, MRSA colonization, dandruff, nail infections and tinea in humans [5].

Tangawisi® products, which are made based not only ginger rhizomes but also other plant organs for medicinal virtue, are prized for their therapeutic value and infections.

There are liquid products used as beverages or products for an enema and solid products which are used as suppositories or chews. They are made from plant organs of certain plants with medicinal properties, the composition, and the proportions vary by manufacturer. But microbiological analysis of some Tangawisi® drinks sold in the marketplaces of Kinshasa in DRC has shown that this type of product Tangawisi® was contaminated by a variety of microorganisms from the raw materials. For the enhancement and improvement of these products, good hygiene must be imposed during manufacturing.

Another route for recovery is the use of essential oils from these plants. Literature provides little data on the antimicrobial activity of essential oils extracted from plant organs used in the manufacture of products Tangawisi® like other aromatic plants.

The objective of this study is to evaluate the antifungal activity of essential oils extracted from some plants of Tangawisi® products against a strain of *Candida albicans*.

Material and Methods

Material

Plant materials

Plant materials of Tangawisi® products were purchased in local markets of Kinshasa and identified by biologists from the University of Kinshasa. They were *Zingiber officinalis* Roscoe (Rhizome) *Securidaca longepedunculata* Fres. (Bark of the root), *Dorstenia psilurus* Welw (Root), *Mondia whitei* (Hook.f.) Skeels (Root), *Monodora myristica* (Gaertn) Dunal (Seed), *Cyperus articulatus* L. (Rhizome) *Xylopi aethiopica* (Dunal) A.Rich. (Clove), *Mangifera indica* Linn. (Bark of the trunk), *Aframomum melegueta* K. Schum. (Seed), *Piper guineense* Schum. And Thonn. (Seed), *Garcinia huiensis* Welw. (Bark of the root), *Acridocarpus congolensis* Sprag (Root), *Heinsia crinita* (Afz) G. Tayl (Root) and *Castanola paradoxa* (Gilg) Schellens (creeper).

Fungal isolate

The culture of fungal isolate of *Candida albicans*, was procured from the Bacteriology Laboratory of University Clinics of Kinshasa.

Chemical and reagents

All solvents used were of analytical grade. Thymol, Eugenol were analytical grade and from Merck.

Methods

Preparation of plant material

The preparation includes sorting, optional washing, cutting and grinding plant material.

Determination of the water and organic volatile component content and the dry matter content

The water content and organic volatile component (WOVC) expressed in percentage, is determined by baking at $105 \pm 1^\circ \text{C}$ according to French standard NF V 03-909 quoted by El Kalamouni [6]. The dry matter content of the sample is obtained from the %WOVC value: % Dry Matter = $100 - \% \text{WOVC}$.

Extraction of essential oils by hydrodistillation

An amount of 100-350 g of a mash of each plant was subjected to hydrodistillation for 2-3 hours. The essential oils obtained were dried by using sodium sulfate and subsequently weighed; they were finally kept in brown bottles kept in dark.

Determination of the essential oil yield

The essential oil yield was calculated using the equation proposed by El Kalamouni [6]:

$$\text{YEO} = \frac{\text{MEO}}{\text{MDP}} \times 100$$

Where YEO: Essential oil yield (%); MEO: Mass of essential oil (g) and MDP: Mass of the dry plant material (g).

TLC analysis

Analytical TLC of solutions was carried out on normal phase Silica gel 60 F₂₅₄ plates (Merck), using Toluene/ Ethyl acetate (97:3) as eluent and visualized in daylight with the anisaldehyde-sulfuric acid reagent. Eugenol, thymol were used as standards. Terpenoids were detected as violet-grey, yellow to violet colored spots [7].

Antifungal assay of some essential oils extracted

Disc-agar diffusion method

Antifungal activity of the oils on *Candida albicans* was assayed by disc agar diffusion method [1] [8]. 20 mL of sterilised Sabouraud's CAF 4% Agar medium (lot 090611204, réf: 610203, Liofilchem, Italy) was taken in each sterile Petri plate (90 mm diameter) and 500 µL 24 h old broth culture of *Candida albicans*, isolated at the Bacteriology Laboratory of University Clinics of Kinshasa, were inoculated, by incorporating into separate sterile Petri dishes. The experiment was performed in 3 replicates. The microbial suspension was concentrated in cells per mL approximately 10^3 - 10^4 . After solidification, sterilized Whatman n°1 filter paper discs of 6 mm diameter were placed in the center of the surface of each culture contained into Petri plates and only one disc was placed. 7.5 µL of each pure essential oil/eugenol to be tested were put on the paper disc and incubated at 30°C for 24 hours. The antifungal activity of the essential oils was determined by assessing the zone of inhibition diameter (ZI).

Broth macro dilution method followed by inoculation in agar medium free of essential oil

The minimal inhibitory concentration (MIC) and the minimal fungicide concentration (MFC) were determined by serial broth macro dilution method followed by inoculation in agar medium free of essential oil proposed by Mann and Markham [9], Bouzouita *and al.* [10] and Bourkhiss *and al.* [11]. Different preparations of 3 mL volume were performed in different test tubes with Sabouraud's dextrose broth (lot 3121113, cat 1805.00, Laboratories CONDA S.A, Madrid, Spain) modified by addition of 0.15% agar, inoculum ($120 \mu\text{L}$ of 10^3 - 10^4 cells/mL) and pure essential oil/eugenol in order to obtain concentrated preparations at 0, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 1, 1.5, 2.5, and 3.5% (v/v) essential oil/eugenol. After homogenization, the different test tubes were then incubated at 30°C for 24 hours. And then, an inoculating loop of each preparation was inoculated by streaking respectively at the center of a sterile petri dish in free-oil Sabouraud's CAF 4% agar medium in 3 repetitions. The plates inoculated were subsequently incubated at 30°C for 24 hours.

The fungistatic or fungicide power

The fungistatic or fungicide power of an essential oil on *Candida albicans* is determined by calculating the ratio MFC/MIC [12].

Results and discussion

Yield extraction of Essential oils by hydro distillation

Extraction with hydrodistillation made from valuable organs

of different plants used in the manufacture of *Tangawisi*® products led to obtaining or not the essential oil with varying

yields. Table 1 lists, for each plant, the organ used and essential oil yield obtained.

Table 1: List of plants essential oil extraction and their average yields

N°	Scientific name	Use organ	Essential oil yield (%)
1	<i>Xylopiya aethiopica</i> (Dunal) A.Rich.	Clove	1.23±0.47
2	<i>Dorstenia psilurus</i> Welw.	Root	0.00
3	<i>Zingiber officinalis</i> Roscoe.	Rhizome	1.31±0.03
4	<i>Mangifera indica</i> Linn.	Bark of the trunk	0.16±0.01
5	<i>Piper guineense</i> Schum. et Thonn.	Seeds	4.94±2.55
6	<i>Mondia whitei</i> (Hook.f.) Skeels	Root	0.00
7	<i>Monodora myristica</i> (Gaertn.) Dunal.	Seeds	1.57±0.15
8	<i>Garcinia huillensis</i> Welw.	Root	0.0000
9	<i>Cyperus articulatus</i> L.	Rhizome	0.59±0.02
10	<i>Securidaca longepedunculata</i> Fres.	Racine	1.07±0.27
11	<i>Aframomum melegueta</i> K.Schum.	Seeds	0.33±0.14
12	<i>Acridocarpus congolensis</i> Sprag.	Bark of root	0.00
13	<i>Castanola paradoxa</i> (Gilg.) Schellens	Root	0.00
14	<i>Heinsia crinita</i> (Afz.) G.Tayl	Root	0.25±0.02

The table 1 above showed that nine plants were identified as essential oil plants that are *Piper guineense*, *Monodora myristica*, *Zingiber officinalis*, *Xylopiya aethiopica*, *Securidaca longepedunculata*, *Cyperus articulatus*, *Aframomum melegueta*, *Mangifera indica* and *Heinsia crinita*.

It should be noted that hydro distillation of all plants listed above gave a less dense essential oil than its hydrolat except hydro distillation of barks of *S. longepedunculata* which led to obtaining oil denser than hydrolat. This phenomenon is probably explained by the presence of methyl salicylate at

large proportion in the essential oil of *S. longepedunculata* that makes this essential oil does not drag on the surface of hydrolat [13-15]. A small portion of methyl salicylate are dissolved in water and a large part form an oily liquid (oil of wintergreen) at the container bottom. It is this oily liquid which was harvested and quantitated.

Characterization of essential oils extracted by thin layer chromatography

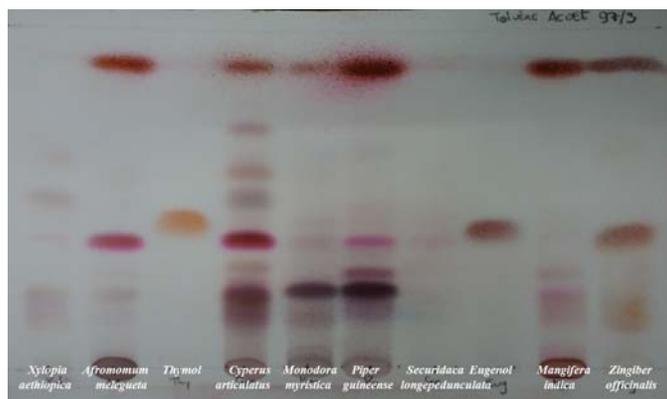


Fig 2: TLC chromatogram of essential oil from aromatic plants of *Tangawisi*® developed with toluene/ethyl acetate 97:3 (v/v) and visualized in daylight with the anisaldehyde-sulfuric acid reagent.

TLC analysis showed that each essential oil had a characteristic chromatographic profile. By comparison with standards, it was showed that thymol is absent in the essential oil studied and eugenol is only present in the essential oil of *Zingiber officinalis*. Essential oils consist of several chemical

compounds having different functional groups [16].

Antifungal assay

The results were summarized in Table 1 as a mean of tree replications.

Table 1: ZI diameter, MIC, MFC, Ratio MFC/MIC and essential oils Power against *C. albicans*

Essential oils	ZI diameter (mm)	MIC (% v/v)	MFC (% v/v)	Ratio MFC/MIC	Essential oil Power
<i>Piper guineense</i>	6	0.5	ND	>5	Fungistatic
<i>Cyperus articulatus</i>	9.33±0.62	0.5	2.5	5	Fungistatic
<i>Securidaca longepedunculata</i>	15.17±0.47	0.05	0.075	1.5	Fungicide
<i>Xylopiya aethiopica</i>	11.33±1.43	0.075	0.5	6.7	Fungistatic
<i>Zingiber officinalis</i>	19±1.22	0.075	0.25	3.33	Fungicide
<i>Monodora myristica</i>	6	0.5	1	2	Fungicide
Eugenol	26±1	0.05	0.05	1	Fungicide

Disc-agar diffusion method

Highest zone inhibition was recorded in eugenol used as positive control followed by *Z. officinalis* oils, *S. longepedunculata*, *X. aethiopica*, *C. articulatus*, *M. myristica* and, *P. guineense*.

The wide spectrum of antimicrobial activity is based on the measure of the diameter of the inhibition halo [17]. Eugenol and *Z. officinalis* oils exert a moderate inhibitory effect, *S. longepedunculata* and *X. aethiopica* oils exert a slight inhibitory effect while *C. articulatus*, *P. guineense* and *M. myristica* showed nul effect.

The *Candida albicans* sensitivity is due to the presence of antifungal constituents of these essential oils assets. The importance of the zone inhibition diameter is not sufficient to characterize the anticandida activity of essential oils. Others methods can be used for determining the inhibitor or lethal activity of essential oils.

Broth macro dilution method followed by inoculation in agar medium free of essential oil

The highest concentration of essential oil that does not manifest the growth in a broth medium (absence of cloudy) is considered minimal fungistatic concentration or minimal inhibitory concentration (MIC) [10]. Minimum inhibitory concentration values recorded are 0.05% in eugenol and *S. longepedunculata* oil, followed by 0.075% in *Z. officinalis* and *X. aethiopica* oils and 0.5% in *P. guineense*, *C. articulatus* and *M. myristica*. Although the zone inhibition diameter is not important around the paper disk, an essential oil may, at a certain concentration, manifest an inhibitory activity.

The high concentration of essential oil that does not allow the colonies formation of *Candida albicans* in a Petri dish in Sabouraud's CAF agar 4%, free of essential oils, is considered minimal fungicide concentration (MFC) or lethal concentration [10] [18]. MFC values are 0.05% in eugenol, 0.075% in *S. longepedunculata* oil, 0.25% in *Z. officinalis* oil, 0.5% in *X. aethiopica* oil, 1% in *M. myristica* oil, 2.5% in *C. articulatus* oil and above 3.5% in *P. guineense* oil.

In the range of concentrations chosen for this experiment, only *P. guineense* oil did not damage the cells of *C. albicans* and did not prevent the resumption of cells multiplication. The action of this essential oil is not lethal but inhibitory. On the contrary, the eugenol and essential oils of *C. articulatus*, *S. longepedunculata*, *X. aethiopica*, *Z. officinalis* and *M. myristica* have caused irreversible damage to the *C. albicans* cells.

The fungistatic or fungicide power

The power of an essential oil is said fungicide when the ratio MFC/MIC is less than or equal to 4; by against, when this ratio is greater than 4, power is said fungistatic [12] [19]. According to these results, essential oils extracted from plants of Tangawisi products carry two types of power on the *Candida albicans* cells. The fungistatic power, responsible for reversible cellular damages, is caused by *P. guineense*, *C. articulatus*, and *X. aethiopica* oils; on the contrary, the fungicide power which is accompanied by irreversible cellular damages is caused by eugenol, *S. longepedunculata*, *Z. officinalis* and *M. myristica* oils.

The anticandida activity of essential oils is mainly due to its chemical profile. Cox and al. [20] reported that exposure of *Candida albicans* cells to the minimum fungicide concentration of *Melaleuca alternifolia* oil, having in its chemical constitution monoterpene compounds, has as effect

the loss of cellular integrity which is manifested by inhibition of cell respiration, by alteration of cell structure membranes making them permeable and leakage of intracellular potassium ions. Under these conditions, the cells lose their vitality and die. Most studies on mechanism action of essential oils have focused on their effects on cell membranes. In fact, the active compounds attack the cell and the cytoplasmic membranes, thereby affecting the permeability and the release of intracellular components, also interfere with the membrane function [21-24].

The difference in the chemical composition of each essential oil extracted from plants of Tangawisi® products explains the difference in susceptibility of *C. albicans* cells to the effects of different essential oils. Chemical compounds with antifungal potential are known like phenolic compounds; their presence in essential oils of *C. articulatus*, *S. longepedunculata* (methyl salicylate, major component that is only visible in UV light at 254 nm), *X. aethiopica*, *Z. officinalis* (eugenol) and *M. myristica* gives them antifungal properties that depend on the nature and proportion of antifungal active (compound), majority and minority compounds synergistic effect of the essential oil considered [5, 25].

Conclusion

The plants used in the manufacture of Tangawisi® products contain organic volatile compounds, especially essential oils, responsible for the flavor and odor and extracte by the hydro distillation technique. TLC method has been established, in a qualitative way, the difference in the chemical composition of different essential oils extracted from these plants. Thymol is not on the list of constituents of the essential oil, eugenol, on the order hand, is a part of the essential oil constituents of *Zingiber officinalis* list. The presence of certain major constituents nevertheless remains an intrinsic characteristic related to plant and characterizes its chromatographic profile. Essential oils extracted from plants of Tangawisi® products demonstrate antifungal activity on a strain of *C. albicans*, which is dependent on the type and concentration of essential oil considered. The presence of phenolic compounds such as eugenol in the essential oil of *Z. officinalis* and methyl salicylate in *S. longepedunculata* oil gives them a significant fungicidal power against the strain of *C. albicans*. The presence of essential oils in these plants justifies their use in the manufacture of Tangawisi® products in particular and in traditional African pharmacopeia in general. The use of essential oils extracted from plant organs of Tangawisi® products may be a better way to value these plants in food, pharmaceutical, medical and cosmetic industries but further studies are needed.

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