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Antibacterial and antiplasmodial potentials of essential oils from two plants of Tangawisi products: *Zingiber officinalis* Roscoe and *Monodora myristica* (Gaertn) Dunal

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Abstract
This work aimed to highlight antibacterial and antiplasmodial potentials of *Zingiber officinalis* and *Monodora myristica* essential oils. The extraction yields in essential oils were 1.28±0.05% for *Z. officinalis* rhizome and 1.52±0.14% for *M. myristica* seeds. Diameter of the inhibition zone formed as a result of *Z. officinalis* oil is 39.17±1.44 mm, 37.00±4.33 mm, 36.17±6.64 mm and 20.67±2.26 mm respectively for *Salmonella sp, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus* strains; for *M. myristica* oil, 11.00±1.00 mm, 7.83±2.75 mm, 7.67±1.53 mm and 6.00±0.00 mm; and for eugenol used as control, 18.5±1.32 mm, 18.83±1.04 mm, 18.50±0.5 mm and 20.17±3.25 mm. The MIC of *Z. officinalis* oil is 0.25% on *Salmonella sp, Klebsiella pneumoniae* and *Staphylococcus aureus* strains and 0.5% on *Pseudomonas aeruginosa* strain; the MIC of *M. myristica* oil is 0.1% on *Salmonella sp* and *Staphylococcus aureus* strains, 0.25% on *Pseudomonas aeruginosa* strain and 0.5% on *Klebsiella pneumoniae* strain and it is 0.05% for eugenol on all bacterial strains. The MBC of *Z. officinalis* oil is 0.5% on *Staphylococcus aureus* strain, 1% on *Salmonella sp* and *Klebsiella pneumoniae* strains and higher than 3.5% on *Pseudomonas aeruginosa* strain; the MBC of *M. myristica* oil is higher than 3.5% on all strains; for eugenol, the MBC is 0.1% on *Staphylococcus aureus* strain and 0.5% on *Salmonella sp, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains. *Z. officinalis* oil has a bactericide action on *Salmonella sp, Klebsiella pneumoniae* and *Staphylococcus aureus* strains and a bacteriostatic action on *Pseudomonas aeruginosa* strain; *M. myristica* oil has a bacteriostatic action on all bacterial strains. *Z. officinalis* oil make total inhibit completely trophozoites up to the concentration less than 6.25µg.mL^{-1} while *M. myristica* oil make total inhibit completely trophozoites up to the concentration of 12.5µg.mL^{-1} and below this concentration, inhibition become partial.

Keywords: Antibacterial potential, antiplasmodial potential, essential oil, *Zingiber officinalis*, *Monodora myristica*, Tangawisi

Introduction
Infectious diseases and malaria are two major public health problems [1] because they are responsible for many deaths around the world. Indeed therapeutic problems arise today especially with the appearance of Plasmodium strains resistant to synthetic antimalarials [2]. Antibiotics or chemical sprays applied for prevention and treatment in human medicine can unfortunately cause selective pressure leading to the spread of resistant mutants [3].
The richness of plant biodiversity and the knowledge of traditional therapies are likely to open new avenues for antibacterial and antimalarial therapeutics [4].
Ginger scientifically known as *Zingiber officinalis* Roscoe is one of the most important plants with several medicinal, nutritional and ethnomedical values therefore, used extensively worldwide as a spice, flavouring agent and herbal remedy. Traditionally, *Z. officinalis* is used in many medicinal systems to cure a variety of diseases viz, nausea, vomiting, asthma, cough, palpitation, inflammation, dyspepsia, loss of appetite, constipation, indigestion and pain [5-7].
Ethnomedically, *M. myristica* is used to treat hemorrhoids, stomach-ache and fibrie pain. The seeds are aromatic and are employed after grinding to a powder as condiments in food providing a flavour resembling that of nutmeg. The seeds are also used as an aromatic and stimulating addition to medicines and to snuff. When pulverized, the kernel is used to prepare pepper soup as stimulant to relieve constipation and control passive uterine hemorrhage in women immediately after child birth [8-10].

Natural agents possessing biological properties have the advantage of being readily accepted by consumers, as they are considered natural [11]. Many medicinal plants contain active compounds which are able to inhibit microbial growth [12] and trophozoites growth [4, 13].

Plants yielding essential oils and other extracts have gained attention and scientific interest as sources of natural products [14] because of theirs various biological and pharmacological properties [3, 14-15].

Therefore, there has been a growing considerable interest to identify new sources of safe and inexpensive antibacterial and antiplasmodial potential of natural origin.

The aim of this study is to highlight antibacterial and antiplasmodial potentials of essential oils extracted from rhizomes of *Zingiber officinalis* and seeds of *Monodora myristica*, two plants of Tangawisi products.

Material and methods

Material

Plant materials

The plant materials consist of two plants organs, the raw material of Tangawisi products: *Zingiber officinalis* Roscoe (Rhizome) and *Monodora myristica* (Gaertn) Dunal (Seed).

Microbial strains

Clinical bacterial strains of *Salmonella sp*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were procured from the Bacteriology Laboratory of the National Institute for Biomedical Research (INRB) and *Klebsiella pneumoniae* from the Bacteriology Laboratory of University Clinics of Kinshasa.

Clinical strain of *Plasmodium falciparum* was come from child blood less than 5 years age.

Methods

Extraction of essential oils by hydrodistillation and Determination of the essential oil yield

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.

The essential oil yield was calculated using the equation:

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

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

Antibacterial assay

Disc-agar diffusion method

Antibacterial activity of the two essential oils was determined by the disc agar diffusion method proposed by [17] and [18]. 20 ml of the sterilized Mueller Hinton agar medium (lot 070414206, Ref. 610110, Liofilchem, Italy) was taken in each sterile Petri plate (90 mm diameter) and 0.5 ml of 24 hours old broth culture of each bacterial strains were inoculated, by incorporating into separate sterile Petri plate. The experiment was performed in 3 replicates. The bacterial suspensions were concentrated in cells per ml approximately 10^6-10^8. After solidification, sterilized Watman 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 37 °C for 24 hours. The antibacterial activity of each essential oil/eugenol was determined by assessing the zone of inhibition diameter (ZI). Statistical analyses were performed using a one-way analysis of variance. A probability value of $p < 0.05$ was considered significant.

Broth macro dilution method followed by inoculating on agar medium free of essential oil

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined by serial broth macro dilution method followed by inoculation in agar medium free of essential oil [19-21]. Different preparations of 3 ml volume were performed in different test tubes with Soybean trypticase broth (lot 092011206, ref: 610053, Liofilchem, Italy) modified by addition of 0.15% agar, inoculum (120 µL of 10^6-10^8 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) of essential oil/eugenol. After homogenization, the different test tubes were then incubated at 37 °C for 24 hours. And then, an inoculating loop of each preparation was inoculated by streaking respectively at the center of a sterile Petri plate in free-oil Mueller Hinton agar medium for bacterial strains in 3 repetitions. The Petri plates inoculated were subsequently incubated at 37 °C for 24 hours.

Bacteriostatic or bactericidal properties

The bacteriostatic or bactericidal power of each essential oil on bacterial strains is determined by calculating the ratio MBC/MIC [20-22].

Antiplasmodial potential of essential oils

The antiplasmodial activity was determined by the in-vitro microtechnique method proposed by Rieckmann *and al.* (1978) cited by [23]. The essential oils were prepared in a solution of 100% methanol/dimethylsulfoxide at 800µg.mL^{-1} respectively. The control used, quinine (commercial solution) was emulsified in 50% of methanol solution. On the well lines of a 96-well sterile microplate, 50 µL of each solution was successively diluted in half to the 7th dilution with 50 µL of methanol, so that the concentration of 800 µg.mL^{-1} decreases to 6.25 µg.mL^{-1}. These dilutions were carried out in 3 replicates in aseptic conditions under a vertical laminar flow hood. Microplates thus impregnated were placed in the oven at 37 °C until complete drying. *Plasmodium falciparum* (clinical strain) of a child less than 5 years age was centrifuged at 1600 rpm for 10 minutes; the resulting globular pellet was diluted 10 times with RPMI 1640 to glutamine medium. Fifty microliters of this mixture were distributed in each well of microplates previously impregnated. Subsequently, the plates were placed in anaerobic conditions at 37 °C for 48 hours. The contents of each corresponding well were spread in a thick drop on a slide. The preparations on slides were stained with 10% Giemsa in buffered water (pH = 7.2) for 10 minutes, rinsed with tap water and dried in atmospheric air. The preparations on slides were observed under the objective 100 of optical microscope at the immersion oil. For each well, trophozoites and schizonts were counted. The percentage of maturation was calculated using the following formula:

% Maturation = (S/T) × 100

Where, S = Number of schizonts in well tested; T = Number of trophozoites in the tested well.

And percentage inhibition was deduced from % maturation, the formula becomes:

% Inhibition = 100 - % Maturation

The average Inhibition percentages were processed using the Excell 2010 software to generate graphs of antiplasmodial activity of both oils and quinine.

Results and discussion

Yield extraction of the two essential oils by hydro distillation

Extraction with hydro distillation made from valuable organs of both plants used in the manufacture of Tangawisi products led to the production of the essential oil with varying yields. Table 1 gives, for each plant, the organ used and the average yield of essential oil obtained.

Table 1: Name and Organ used of both plants and Average yield of extraction (%).

Plant name	Organ used	Essential oil yield (%)
Zingiber officinalis	Rhizomes	1.28±0.05
Monodora myristica	Seeds	1.52±0.14

The yields of *Zingiber officinalis* and *Monodora myristica* essential oils obtained by hydro distillation were 1.28% and 1.52%, respectively.

Antibacterial assay

Disc-agar diffusion assay

Bacteria were observed after incubation on growth media. The growth-inhibitory effects of the two essential oils and eugenol against four bacteria are shown in Table 2. Both essential oils possessed antibacterial activities against all the bacteria tested in different levels.

Table 2: Zone inhibition diameter (ZI) formed under effects of both essential oils and eugenol on four bacterial strains.

Essential oils /Eugenol	Zone inhibition diameter (mm)			
	Salmonella sp	Pseudomonas aeruginosa	Klebsiella pneumoniae	Staphylococcus aureus
Z. officinalis	39.17±1.44[a]	37.00±4.33[a]	36.17±6.64[a]	20.67±2.26[a]
M. myristica	11.00±1.00[c]	7.83±2.75[c]	7.67±1.53[c]	6.00±0.00[b]
Eugenol	18.50±1.32[b]	18.83±1.04[b]	18.50±0.50[b]	20.17±3.25[a]

Numbers in column following by same letter are not significantly different (p-value<0.05).

In considering the inhibition zone, both essential oils exerted greater antimicrobial effects against the Gram-negative bacteria than against the Gram-positive bacteria. The wide spectrum of antimicrobial activity is based on the measure of the diameter of the inhibition halo [24]. *Z. officinalis* oil exerts a greatly inhibitory effect, Eugenol a moderate inhibitory effect, and *M. myristica* a slight inhibitory effect. *Z. officinalis* oil exerted a stronger antibacterial effect than *M. myristica* oil. On the contrary, eugenol exerted greater antimicrobial effects against the Gram-positive bacteria than against the Gram-negative bacteria. The importance of the zone inhibition diameter is not sufficient to characterize the antibacterial activity of essential oils. Others dilution methods can be used for determining the inhibitor or lethal activity of essential oils.

Broth macro dilution assay followed by inoculating on agar medium free of essential oil and essential oil power

The results of MIC, MBC, ratio MBC/MIC and bacteriostatic or bactericide power of both essential oils and eugenol used than reference are shown in tables 3, 4 and 5.

MIC is the highest concentration of essential oil that does not manifest the bacteria growth in a broth medium (absence of cloudy) [20] or the lowest concentration of the essential oil capable of inhibiting the growth of the challenging microorganism [19]. The MIC of *Z. officinalis* oil is 0.25% for *Salmonella sp*, *Staphylococcus aureus* and *Klebsiella pneumonaie* and 0.5% for *Pseudomonas aeruginosa*; the MIC of *M. myristica* oil is 0.10% for *Salmonella sp* and *Staphylococcus aureus*, 0.25% for *Pseudomonas aeruginosa* and 0.5% for *Klebsiella pneumonia*. For the reference, eugenol, the MIC is 0.005% for the four bacterial strains.

MBC is the high concentration of essential oil that does not allow the colonies formation of bacterial strains in a Petri dish in Mueller Hinton agar, free of essential oils [22]. The MBC of *Z. officinalis* oil is 0.5% for *Staphylococcus aureus*, 1.00% for *Salmonella sp* and *Klebsiella pneumonia* and higher than 3.5% for *Pseudomonas aeruginosa*; the MBC of *M. myristica* oil is higher than 3.5% for all bacterial strains used in this study. The MBC of eugenol is 0.1% for *Staphylococcus aureus* and 0.5% for *Salmonella sp*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

The power of an essential oil is said bactericide when the ratio MBC/MIC is less than or equal to 4; by against, when this ratio is greater than 4, power is said bacteriostatic [20, 22]. *Z. officinalis* oil exerts bactericide action on *Salmonella sp*, *Staphylococcus aureus* and *Klebsiella pneumoniae* and bacteriostatic action on *Pseudomonas aeruginosa* while *M. myristica* oil exerts bacteriostatic action. Eugenol power is bactericide against *Staphylococcus aureus* and bacteriostatic against three gram-negative bacterial strains.

Pseudomonas aeruginosa resistance is well known against all antimicrobial actives because of his capacity to form biofilm [25-29]. In addition, *S. aureaus* was shown to be the most susceptible strain as found [26] that most Gram-positive bacteria were more sensitive to inhibition by plant essential oils than the Gram-negative bacteria.

It was also hypothesised that monoterpenes and sesquiterpenes with phenolic hydroxyl groups can form hydrogen bonds with the active sites of the target microorganisms and contributes to the overall antimicrobial effects of the essential oils. These chemical components exert their toxic effects against microorganisms by distrupting bacterial integrity [28, 30].

Most of the antimicrobial activity in essential oils derived from spices and culinary herbs is believed to derive from phenolic compounds, whereas other constituents are believed to contribute little to the antimicrobial effects [31-35].

Thus, the antimicrobial activity of *Z. officinalis* essential oil tested in this study may be the result of the presence of high levels of eugenol [36]. Sure enough, [37] reported that eugenol may provoke cellular wall damage result in membrane ATP synthetase activty inhibition in bacteria.

These results indicate that both *Z. officinalis* and *M. myristica* oils exert strong antimicrobial effects at low MICs. While both can be used as natural antimicrobial substances, *Z. officinalis* oil displays a stronger effect than *M. myristica* oil.

Table 3: MIC, MBC, Ratio MBC/MIC and essential oil Power of *Zingiber officinalis* against four bacterial strains.

Bacterial strains	MIC (% v/v)	MBC (% v/v)	Ratio MBC/MIC	Essential oil Power
Salmonella sp	0.25	1.00	4	Bactericide
Staphylococcus aureus	0.25	0.5	2	Bactericide
Pseudomonas aeruginosa	0.5	ND	>4	Bacteriostatic
Klebsiella pneumoniae	0.25	1.00	4	Bactericide

ND: No determined because the concentration is higher than 3.5%.

Table 4: MIC, MBC, Ratio MBC/MIC and essential oil Power of *Monodora myristica* against four bacterial strains.

Bacterial strains	MIC (% v/v)	MBC (% v/v)	Ratio MBC/MIC	Essential oil Power
Salmonella sp	0.10	ND	>4	Bacteriostatic
Staphylococcus aureus	0.10	ND	>4	Bacteriostatic
Pseudomonas aeruginosa	0.25	ND	>4	Bacteriostatic
Klebsiella pneumoniae	0.5	ND	>4	Bacteriostatic

Table 5: MIC, MBC, Ratio MBC/MIC and Eugenol Power against four bacterial strains.

Bacterial strains	MIC (% v/v)	MBC (% v/v)	Ratio MBC/MIC	Eugenol Power
Salmonella sp	0.05	0.5	10	Bacteriostatic
Staphylococcus aureus	0.05	0.1	2	Bactericide
Pseudomonas aeruginosa	0.05	0.5	10	Bacteriostatic
Klebsiella pneumoniae	0.05	0.5	10	Bacteriostatic

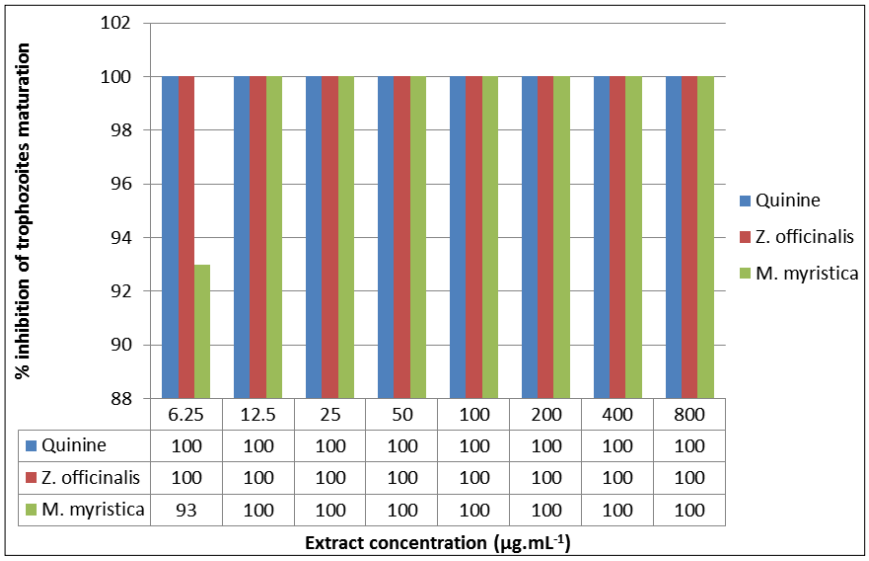
Antiplasmodial assay

Antiplasmodial activity is manifested by inhibition of trophozoites maturation as described in figure 1. In the concentrations range of different extracts used (800-6.25 µg.mL^{-1}), trophozoites inhibition is total for quinine and *Zingiber officinalis* essential oil extracts. Until 6.25 µg.mL^{-1}, essential oil of *Zingiber officinalis* exerts antiplasmodial activity like quinine. On the contrary, essential oil extract of *Monodora myristica* exhibit total inhibition of trophozoites maturation up to 12.5 µg.mL^{-1}. Above this concentration, inhibition becomes partial like showed [13] with *Xylopia aethiopica* and *Cyperus articulatus* essential oils.

The extracts are considered active when the inhibitory concentration is less than 5 µg.mL^{-1} [4]. The IC_{50} for three extracts could be estimated lower than 6.25 µg.mL^{-1}. The both essential oils content active compounds against trophozoites and can be used for malaria treatment.

[38] Showed that two sesquiterpenes, corymbolone and mustakone, isolated from the chloroform extract of the rhizomes of *Cyperus articulatus*, exhibited significant anti-plasmodial properties. Mustakone was approximately ten times more active than corymbolone against the sensitive strains of the *Plasmodium falciparum*. Sesquiterpenes present in both essential oils [39-40] contribute actively to their antiplasmodial potentials.

The mode of antiplasmodial action of essential oils is not known. Taking into account of plasmodial targets of convential antipaludics [41], the hypothesis of action mode similar to the mode of antimicrobial action [42-44] could be considered in *Plasmodium falciparum*.



	6.25	12.5	25	50	100	200	400	800
Quinine	100	100	100	100	100	100	100	100
Z. officinalis	100	100	100	100	100	100	100	100
M. myristica	93	100	100	100	100	100	100	100

Fig 1: Inhibition of trophozoites maturation by extracts of quinine and both essential oils.

Conclusion

Z. officinalis and *M. myristica* essential oils hold antibacterial and antiplasmodial potentials and can be used as natural antibacterial and antiplasmodial substances to the treatment of bacterial infections and malaria. Depending on the microorganisms present in the food, both essential oils can be used as natural food preservatives. The use of essential oil of both plant organs is one of the ways to enhance the value of plants used in the manufacture of Tangawisi products.

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