Full Length Research Paper

Galenic formulation and antimicrobial activities of tablets made from essential oil of *Lippia multiflora* Moldenke (Verbenaceae)

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Accepted 11 February, 2013

Essential oil-containing tablets made from essential oil of *Lippia multiflora* Moldenke have been developed with an intention to treat buccal and pharyngeal infections after perlingual administration. Tablets were accomplished with Arabic gum, mannitol and *L. multiflora* essential oils (2.5% w/w) and tested for antimicrobial activity using agar dilution techniques. The tablets were tested against strains from buccal and pharyngeal flora like *Streptococcus β hemolyticus A*, *Streptococcus α hemolyticus*, *Staphylococcus aureus*, *Staphylococcus non aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and fungi *Candida albicans*. Galenical and biogalenical controls proved to be satisfactory. Essential oil-containing tablets possessed significant antimicrobial effect. Gram-positive bacteria and fungi were more sensitive to test tablets than Gram-negative bacteria. The essential oil of *L. multiflora* formulated in tablets retained its antimicrobial activity against oral and pharyngeal pathogen.

Key words: *Lippia multiflora*, essential oil, essential oil-containing tablets, antimicrobial.

INTRODUCTION

Serious infections caused by bacteria that have become resistant to commonly used antibiotics have become a major global healthcare problem in the 21st century. In the developing countries, bacterial infections are still the main cause of deaths (Iwu et al., 1999). Down the ages, essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases (Tepe et al., 2004). According to the World Health Organization (WHO), about 80% of the people in developing countries rely primarily on medicinal plants for their primary health care (Wood-Sheldon et al., 1997). Medicinal and aromatic plants are widely used as medicine and constitute a major source of natural organic compounds. It has long been acknowledged that some plant essential oils exhibit antimicrobial properties and it is necessary to investigate these plants scientifically. Essential oils are potential sources of novel antimicrobial compounds, especially against bacterial pathogens (Azizi et al., 2009). *Lippia multiflora*, a member of the Verbenaceae family, is an aromatic and medicinal plant of West Africa (Adjanohoun and Aké, 1979). African traditional medicine healers use their leaves as tea and in the treatment of malaria, hyper-

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A number of studies have demonstrated the antimicrobial properties of *L. multiflora* oils and its major components against a wide range of microorganisms, the same microorganisms of the buccal flora (Bassole et al., 2003; Oladimeji et al., 2008; Kanko et al., 2004; Bassolé et al., 2003). A number of studies have demonstrated the antimicrobial properties of *L. multiflora* oils and its major components against a wide range of microorganisms, the same microorganisms of the buccal flora (Bassole et al., 2003; Oladimeji et al., 2004; Oussou et al., 2004; Kunle et al., 2003; Pelissier et al., 1994).

Our research focused on developing an oral pharyngeal formula processed into tablets containing this essential oil of *L. multiflora* and to evaluate its antimicrobial activity against oral clinical isolates of Gram positive (*Staphylococcus aureus*, *Staphylococcus* negative-coagulase, Streptococque A, Streptococque α, *Enterococcus faecalis*) and Gram negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosae*) bacteria, in addition to a pathogenic fungus, *Candida albicans*. The main aim of this study was therefore to demonstrate whether the essential oil of *L. multiflora* when associated with prior judiciously chosen excipients within a galenical formulation could retain its antimicrobial activity.

### MATERIALS AND METHODS

**Collection of plant material and extraction process of essential oils**

The leaves of *L. multiflora* were collected in Boundiali, Northern Côte d’Ivoire, in March, 2006. Taxonomic identification of the plant was performed by a Botanist, Prof. Ake Assi of the Centre National de Floristique d’Abidjan (CNF). *L. multiflora* was dried for 10 days at 28 and 30°C in the drain board of the laboratory. Air-dried plant material (100 g) of *L. multiflora* was extracted by hydro-distillation for 2 h in a modified Clevenger-type apparatus (yield 0.5% v/w). Three kinds of distillations were brought to the boiler for 2 h, using air-dried vegetal material (100 g) in 1 L of water; the whole mixture was put in a 2 L balloon, topped by a 60 cm long airstream and connected to a refrigerator. The essential oil thus obtained was protected from light and stored at 4°C till further use.

**Excipients**

All excipients used for the galenical formula were obtained from Cooper Pharmaceuticals, (France).

**Microbial strains**

A panel of twenty six common pathogenic organisms was used for antibacterial tests (Table 1), which includes gram-positive bacteria, gram-negative bacteria and fungi. These clinical isolates were obtained from oral pharyngeal samples of the patients suffering from pharingitis, from the external Ear Nose and Throat (ENT) Department, CHU-Cocody, Côte d’Ivoire. All strains were purified by three successive streaking and re-isolation on Mueller Hinton agar or Saboraud chloramphenicol agar. The purity and identity were confirmed by standard bacteriological methods (Feron, 1994). Media: Nutrient broth No 2, pH 7.4; nutrient agar, pH 7.4; malt extract broth, pH 5.6; Mueller Hinton agar (MHA) are all products from Bio-Rad Laboratories, (France) used for the experiment.

**Formulation of essential oil-containing tablets**

Some pre formulation tests carried out and taken into account organoleptic features, colour and consistency helped constitute a basic formula. A unique permanent formula was adopted so that the final concentration in essential oil of *L. multiflora* could stand to 150 µg/ml. Pounded in a Chinese made mortar, a pasty mixture was obtained and prepared in water with all excipients. The essential oil was then added to this pasty mixture, homogenized and allowed to solidify. After 3 to 5 days, solidified homogeneous paste was chopped off into pieces of 1 g unit.

**Mascroscopical and biogalenical tests**

Ten volunteers were given the tablets and questioned for their opinion on its colour, taste and odour. The pH value was set by using pH meter. Ten gram (10 g) of tablets were weighed and dissolved in 90 ml of germ free distilled-water. After filtration, the homogeneous solution thus obtained allowed the identification of

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**Table 1. Profile of resistance of the Cocci positive Gram to antibiotics.**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OXA&lt;sup&gt;R&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. aureus</em> (N=3)</td>
<td>0</td>
</tr>
<tr>
<td><em>S. non aureus</em> (N=1)</td>
<td>0</td>
</tr>
<tr>
<td>Streptococque A (N=1)</td>
<td>-</td>
</tr>
<tr>
<td>Streptococque α (N=8)</td>
<td>-</td>
</tr>
<tr>
<td><em>E. faecalis</em> (N= 5)</td>
<td>-</td>
</tr>
</tbody>
</table>

OXA: Oxacilline 5 µg; AM: amoxicilline 20 µg; CTX: cefotaxime 30 µg; CAZ: ceftazidine 30 µg; GM: gentamycine 15 and 500 µg; E: erytromycine 15 UI; L: lincomysine 15 µg; VAN: vancomycine 30 µg; LVX: levofloxacine 5 µg; R: resistant; Rn: low natural resistance; Rn: natural resistance.
the pH rate; five measurements were performed to achieve this stage. The biogalenic test consisted in determining the disintegrative duration. This was realized with an Erweka set of apparatus equipped with an oscillating basket. A unit quantity of 10 g of tablets and a disc were put in the different 6 tubes of the oscillating basket apparatus, then it was immersed in a 1 liter Becher flask by using a method of to and fro vertical movements (28 per minute with an amplitude of 5 cm). The final stage of disintegration was reached at a point where no sediment (remaining paste) was observed on the grating. The disintegration tests were immediately carried out upon tablets just after its preparation (Day 0) and also on each consecutive day after its preparation (that is, Day 1 to 7).

Antibacterial tests

The agar dilution method was used to assess the antimicrobial activities of the essential oil-containing tablet. One series of dilutions of tablets was prepared in peptoned thinner previously buffered with tween (monopotassium phosphate: 3.5 g; disodium phosphate: 7.25 g; sodium Chloride: 4.3 g; peptone 1; tween 80: 3 g; water qsp: 1000 ml). Each concentrations of tablets incorporated in Mueller Hinton agar (bacteria) or Sabouraud-chloramphenicol (fungus) were 18.75, 30, 37.5 and 60 µg/ml. The diluent was also used as the negative control. A suspension of the organism is prepared to be tested equivalent to $10^6$ cfu/ml (Oussou et al., 2008), and 10 µl of this suspension is placed on each of the series of plates using a micro-pipette (final inoculum were $10^5$ cfu/spot). Four different bacterial isolates (plus quality control organisms) can be tested simultaneously on each agar plate. For fungi, two spot were placed on each of the series of plates containing Sabouraud-chloramphenicol. Two fungi have been tested simultaneously on each agar plate. The plates were incubated for 18 to 24 h at 37°C (bacteria) and 72 h at 30°C (fungi). Amoxicillin (20 µg) was used as positive controls for bacteria and Amphotericin B (100 µg) for fungus.

Each test was performed in triplicate and repeated thrice. Growths in plates were estimated in term of inhibition percentage reckoned from a proportion of 100% survival in the reference plates of growth control. The calculation method of the inhibition percentage of each bacterium at various tested concentrations could be summed up by the following formula:

$$I = 100 - \frac{n}{N} \times 100$$

Where I: Inhibition of microbacterial isolates (in percentage %), N: number of cultured microbacterial isolates, n: number of positively grown microbacterial.

RESULTS AND DISCUSSION

Macroscopical and biogalenic tests

The tablets formula consisted of 40% pulverized Arabic gum, 40% of mannitol, 250 µl of L. multiflora essential oil and distilled water qsp 10 ml. The tablets displayed a homogeneous particularity, a slightly sweet taste and citronella like fragrance, with an average pH of 7.4. The biogalenic results (desintegration duration) carried out upon the tablets are presented in Figure 1, and the tablets disintegration curve testified the evolution from day 0 to day 7. The curve stayed stationary with dissolution duration of the tablets less than 1 min from day 0 to day 7; from day 2 to day 5, this duration altered...
Table 2. Profile of resistance of negative the Gram bacilli to antibiotics.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>AM&quot;</th>
<th>AMC&quot;</th>
<th>AN&quot;</th>
<th>TIC&quot;</th>
<th>CIP&quot;</th>
<th>CTX&quot;</th>
<th>CXM&quot;</th>
<th>GM&quot;</th>
<th>IMP&quot;</th>
<th>PIP&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella pneumoniae</em> (N=2)</td>
<td>Rn</td>
<td>1</td>
<td>1</td>
<td>Rn</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Escherichia coli</em> (N=2)</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> (N=2)</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

AMC: amoxicillin + acide clavulanique 20 + 10 µg; AN: amikacine 30 µg; TIC: ticarcilline 75 µg; CIP: ciprofloxacine 5 µg; CXM: cefadroxyl 30 µg; IMP: imipineme 10 µg; PIP: piperacilline 75 µg.

Table 3. Antibacterial activities upon bacterial clones by the tablets (% of inhibition).

<table>
<thead>
<tr>
<th>Bacterial clones</th>
<th>Dilution of the tablets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2/5</td>
</tr>
<tr>
<td><em>Streptocoque β hemolytique A</em> (N=1)</td>
<td>100</td>
</tr>
<tr>
<td><em>Streptocoque α hemolytique</em> (N=8)</td>
<td>100</td>
</tr>
<tr>
<td><em>S. aureus</em> (N=3)</td>
<td>100</td>
</tr>
<tr>
<td><em>S. non aureus</em> (N=1)</td>
<td>100</td>
</tr>
<tr>
<td><em>E. faecalis</em> (N=5)</td>
<td>100</td>
</tr>
<tr>
<td><em>E. coli</em> (N=2)</td>
<td>100</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> (N=2)</td>
<td>100</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> (N=2)</td>
<td>0</td>
</tr>
</tbody>
</table>

Antibacterial tests

Plant essential oil extracts have been used for many thousands of years, especially in food preservation, pharmaceuticals, medicine and natural therapies (Hazzit and Baaliouamer, 2009). It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Essential oils are potential sources of novel antimicrobial compounds (Mitscher et al., 1987), especially against bacterial pathogens.

The antimicrobial profile of essential oil-containing tablets is shown in Table 1. The figures in the table are calculated mean percentage of inhibition. In general, essential oil-containing tablets displayed varying degrees of antimicrobial activity against the tested microorganisms. At the concentration of 37.5 µg/ml, tablets inhibited the growth of S. β hemolyticus A, S. α hemolyticus A, S. aureus, S. non aureus and E. faecalis (Table 2). They also inhibited the growth of all the strains of *E. coli* and *K. pneumoniae* at 60 µg/ml. The tablet had strongest activity against *C. albicans* at 30 µg/ml (Table 3). *Pseudomonas aeruginosa* was the most resistant bacteria since the essential oil-containing tablets showed no inhibition towards it. For tablets, the gram-positive bacteria and fungi *C. albicans* were more sensitive compared to the gram-negative bacteria (Table 4).

The *in vitro* activities tests carried out with the tablets shows an inner considerable antibacterial activity. The *L. multiflora* essential oil-containing tablets have therefore retained their antibacterial properties when probed upon pathogenic bacteria of buccal flora. The results obtained were in perfect comformity with those reported by Pelissier et al. (1994) and Adon (1993), who used *L. multiflora* essential oil-mouth rinse and essential oil-toothpaste, respectively. The galenical formulation was reported to be highly active against isolated microorganisms (bacteria and fungi) of the buccal flora and thus supporting its traditional use in mouthwash in some communities.

from 1 min to 1 min 24 s. While from day 5 to day 7, the disintegration time remained basically constant at 1 min 30 s.

The biogalenical study of the preparation indicated an increase of disintegration duration of tablets during their conservation. This duration rose by 30 s after seven days of their preparation. As disintegration plays an important role in maintaining porosity and hardness of the galenical shape, it was deduced that during the conservation, either the hardness had increased or the porosity had decreased. It might be related to the presence of the Arabic gum which becomes hard in presence of humidity and reduces porosity of the galenical shape by increasing the connections between particles. Thus, it is advisable to keep these tablets in water-tight packaging (bearing a weak humidity rate).
In general, the antimicrobial activity of the essential oils tested was more pronounced against Gram-positive than against Gram-negative bacteria, a general observation derived from studies with essential oils from many other plant spices (Nostro et al., 2000). This generally higher resistance among Gram-negative bacteria could be ascribed to the presence of their phospholipidic membrane, almost impermeable to lipophilic compounds (Nikaido and Vaara, 1985). The absence of this barrier in Gram-positive bacteria allows the direct contact of essential oil's hydrophobic constituents with which they bring about their effect, causing either an increase of ion permeability and leakage of vital intracellular constituents, or impairment of the bacterial enzyme system (Cowan, 1999).

P. aeruginosa was definitively too robust. This resistance is not astounding, for the bacterium is endowed with an intrinsic resistance to biocides because of the nature of its external membrane. The latter consists of lipopolysacharides which build up a barrier impervious to hydrophobic composites. Once it is tested by permeabilising agents, this external membrane's inactive substances against P. aeruginosa become active (Ratledge and Wilkinson, 1988). Noted to be implicated in various infections whose evolutions are more or less acute, depending on the status of the environment, and very often dramatic for acquired immune deficiency sick people (Siqueira et al, 1985), C. albicans fungi turned out to be very sensitive compared to other microorganisms.

The essential oil of L. multiflora originated from Côte d'Ivoire is composed of a majority of oxygenated monoterpenes. Kanko et al. (2004) and Oussou et al. (2004) identified 1,8-cineol, geranial and α-terpinene as its major composites, whereas Pelissier et al. (1998) reported tagetone, myrcène and ipsénone to be its major composites. Antimicrobial properties of the essential oil of L. multiflora are well-known, as well as its composites which are ascribed with strong antibacterial activity. Amvam et al. (1998) asserted that these composites probably would operate in occasional synergistical manner.

The interest of the tablet formulation resides in the fact that the tablet form will allow sustained release time of the essential oil and therefore have the following advantages: Prolonged efficacy, and better adherence because the number of doses administered is reduced, while the administration of the essential oil directly has the following disadvantages of large number of firms that makes compliance difficult and risk of significant toxicity.

Table 4. Inhibitive activity of the tablets upon the C. albicans (% of inhibition).

<table>
<thead>
<tr>
<th>Mycotic clone</th>
<th>Amphotericine B</th>
<th>Dilution of the tablets</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans (N=2)</td>
<td>100</td>
<td>100 100 100 50</td>
</tr>
</tbody>
</table>

With the intention of verifying if a galenical form responding to a perlingual administration with active constituent of L. multiflora essential oil could still keep its antibacterial properties, we ventured on formulating a formula in which the active constituent was interacting with prior judiciously chosen excipients. The essential oil of L. multiflora Mold is a part of these natural substances whose natural antibacterial activity had largely been proved. Its occurrence in the formulation of tablets for therapeutical proposes has shown its efficiency upon all isolated organisms of the oral pharyngeal cavity except P. aeruginosa. Essential oil-containing tablets with essential oil of L. multiflora was found to have significant antibacterial activity and must further be subjected to stability testing and clinical studies before they can be prescribed as phytomedicines in the treatment of oral mucous infections and the oropharynx.

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