



Investigation of antibacterial and antifungal activities of essential oils of *Lippia javanica* and *Lantana camara* (Verbenaceae) harvested in the Haut-Katanga (DR Congo)

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ABSTRACT

Background: Essential oils are volatile compounds characterized by a strong odor, and are generally biosynthesized by aromatic plants as secondary metabolites. This paper aims to extract the essential oils of *Lippia javanica* and *Lantana camara*, and to evaluate their antibacterial, and antifungal activities.

Methods: The aerial parts of *Lippia javanica* and *Lantana camara* were subjected to hydrodistillation to produce the essential oil. The antimicrobial potential was characterized against six microorganisms, signifying three Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*), two Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pneumoniae*) and one fungus

(*Candida albicans*) by the disc diffusion method to determine the inhibition zone (in mm) and dilution method to determine the minimal inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC).

Results: Essential oil extraction was carried out with an average yield of 0.21% for *Lippia javanica*, and 0.11% for *Lantana camara*. The evaluation of the antimicrobial activity showed that *Lippia javanica* essential oil had a moderate inhibitory activity on *Klebsiella pneumoniae*, and *Streptococcus pneumoniae* (MIC: 0.76 mg/mL), on *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (1.50 mg/mL). The *Lantana camara* essential oil showed weak inhibitory activity against all strains tested. By diffusion disk method, it was found that *Klebsiella pneumoniae* was the most sensitive on *Lippia javanica* essential oil with an inhibition diameter, which evolved from 7 mm to 24 mm; followed by *Pseudomonas aeruginosa* (21 mm), *Escherichia coli* (19 mm) and *Streptococcus pneumoniae* (13 mm) at 15 μ L. By means of dilution method, the *Lantana camara* essential oil showed a low activity against *Escherichia coli* (MIC: 1.64 mg/mL), *Klebsiella pneumoniae* (MIC: 1.64 mg/mL), *Staphylococcus aureus* (MIC: 3.28 mg/mL), *Pseudomonas aeruginosa* (MIC: 3.28 mg/mL), and *Candida albicans* (MIC: 3.28 mg/mL) but by disc diffusion method, this oil was slightly inhibitory activity on *Escherichia coli* (10 mm at 15 μ L). For the antifungal activity, the *Lantana camara* essential oil, and Germicide were inactive on *Candida albicans* when tested by the disk method.

Conclusion: The essential oil of *Lippia javanica* showed moderate antibacterial and antifungal activities, while the essential oil of *Lantana camara* showed low activity. The activities of essential oils studied were less than that of the gentamicin and more than the activity of Germicide, with two positive controls used.

Keywords: Antibacterial, Antifungal, Essential oil, *Lippia javanica*, *Lantana camara*.

1. Introduction

Many pathogenic microorganisms are the subject of extensive research in the therapeutic and pharmaceutical fields. With current technology development, the nature of the virus, fungus, or bacteria that men are the host is well known but the measures taken once infected are not well controlled. Some bacterial species are adapted so well to antibiotics that they are fewer and less sensitive [1, 2] and sometimes they become either multidrug resistance or multiresistant or extensively drug-resistant. Hence, the need for new solutions is required; in this case, medicinal and aromatic plants are among the main solutions.

Indeed, plants are a huge source of complex bioactive chemical substances exploited in several industries such as

the cosmetics, agro-food, and pharmaceutical industries [3, 4]. Some plant extracts, including essential oils, are used for their strong bactericidal, virucidal, fungicidal, insecticidal, antidiabetic, antioxidant, and anticancer actions. They are also used in alimentary and cosmetic domains [5-8].

Different aromatic plants, mainly characterized by their odorous chemical substances, are used for their antiseptic and therapeutic activity in traditional medicine [9,10]. The history of aromatherapy was born and, with the advances in science, new active ingredients and pharmacological properties made it possible to make aromatic and medicinal plants as authentic drugs [11]. Thus, essential oils, currently used as food flavorings, are also known to possess antimicrobial and antifungal activities. These biological

activities are the topic of many publications worldwide [1, 12-14].

Lippia javanica is a wild medicinal plant found in South and tropical Africa. It is a very robust and multi-branched woody shrub that can reach 1 to 2 m [15, 16]. The *Lippia javanica* leaves are used in traditional medicine in the treatment of malaria, cough, flu, colds, fever, and diarrhea. These leaves are also used in the treatment of asthma, yellow fever, chronic coughs, and respiratory infections. Skin disorders such as abrasions, bites, and scabies can be treated with leaves of *Lippia javanica*. The decoction of its leaves mixed with *Eucalyptus grandis* leaves is used to treat respiratory infections. Malaria is also treated by the mixing of *Lippia javanica* leaves and *Artemisia afra* leaves [17, 18].

Lantana camara is a flowering ornamental plant belonging to the family Verbenaceae. It is also used as a medicinal plant in the traditional medicinal system and recent scientific studies have emphasized the possible use of *Lantana camara* in modern medicine. *Lantana camara* leaves are used as an antipyretic, antispasmodic agent, and in the treatment of malaria. Its leaves are also used to treat rheumatism, cough, fever, measles, and asthma, but also poultry pox [19]. In recent history, this plant is reported for various medicinal properties especially hepatoprotective effect, antibacterial activity, cytotoxic activity, antifertility activity, antifungal

activity, antiurolithiatic activity, anti-inflammatory activity, antimotility activity, antidiabetic activity, larvicidal activity, antioxidant activity and wound healing activity. Different parts of *L. camara* contain essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, iridoid glycosides, phenylethanoid, oligosaccharides, quinones, saponins, steroids, triterpenes, sesquiterpenoids and tannin as major phytochemical groups [20].

Thus, this work aimed to compare the antibacterial activity of essential oils of *Lippia javanica* and *Lantana camara*, harvested in the Haut-Katanga province (DRC) on *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, and *Staphylococcus aureus* and the antifungal activity of these oils on *Candida albicans*.

2. Material and Methods

2.1. Plant material

Samples of *Lippia javanica* and *Lantana camara* were collected during the dry season in August 2017. Only the aerial parts, i.e. leaves, flowers and fruit, were collected in the early morning. *Lippia javanica* was harvested in the Kashamata locality, on the termitary along the Kafubu River, by Kisimba Kibuye Emile, a botanist from the Geography Department of Lubumbashi University. *Lantana camara* was harvested in the Shindaika Street of Ruashi commune (Fig. 1).



Figure 1. Areas where samples were collected in Haut-Katanga (DR Congo)

2.2. Active substances used

The essential oils tested were extracted from *L. javanica* and *L. camara* leaves by hydrodistillation in the lab of the Chemistry department of the University of Lubumbashi (DR Congo). They were stored at 4 °C in the refrigerator (Liebherr Comfort), after extraction. For comparison purposes, two references, gentamicin (an antibiotic) and Germicide (Commercial product) were used. Indeed, Germicide is a surface disinfectant manufactured by Microscientific Industry Corporation (Canada). It can kill *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and human immunodeficiency virus (HIV-1) at 20 °C in only 3 minutes of contact.

2.3. Test microorganisms

Five pathogenic bacteria were used to evaluate the antibacterial activity of essential oils the synthetic Germicide. These include Gram-negative bacteria: *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae* (ATCC13883) and *Pseudomonas aeruginosa* (ATCC27853) and Gram-positive bacteria: *Staphylococcus aureus* (ATCC25923) and

Streptococcus pneumoniae (ATCC49619). *Candida albicans* (ATCC10231), a filamentous fungus, was used for the evaluation of the antifungal activity of these essential oils.

2.4. Extraction of essential oil

The fresh aerial plant material of each sample (1000-1200 g) was cut into small pieces and hydrodistilled using the Clevenger apparatus for 6 h. The Clevenger-type apparatus consists of a power regulator, the heating mantle with a round bottom flask containing water and aromatic leaves, the apparatus, which returns the hydrosol to the still and maintains the essential oil phase and the condenser. The essential oil was transferred into a stoppered tube and stored in a refrigerator at 4 °C [21-24].

2.5. Assessment of antimicrobial activity by disc diffusion method

The antimicrobial screening of the essential oil was evaluated using the agar disc diffusion method. A sterile saline solution was inoculated with an 18–24 h growth culture of bacterial and *candida* strains, and then adjusted

approximately to 10^6 colony-forming units (CFU)/mL for bacteria and 1×10^3 cells/mL for fungi. The suspension was spread on Petri dishes containing Mueller-Hinton Agar. Then, sterile discs (6 mm in diameter), impregnated with 5-10-15 μ l of the essential oil or the Germicide, were placed on the surface of Petri dishes separately inoculated with different tested strains. An antibiotic, gentamicin, was used as the positive control. Before incubation, all plates were stored in the dark at 4 °C for 2 h, to

allow the diffusion of the oil from disc to medium without microbial growth. Then, the plates were incubated at 28-37 °C for 24 h. The antibacterial and antifungal activities were determined by measuring the diameter of the inhibition zone (mm), formed around the disc [25]. A scale for measuring the antimicrobial oils was issued by Ela *et al.* [26] and Meena and Sethi [27], dividing the zone of the microbial growth inhibition zones into 4 classes (Table 1).

Table 1. Classification of antibacterial activity according to inhibition diameter [26, 27].

Diameter	Classification
$\emptyset \geq 28$ mm of the inhibition zone	Heavily inhibitory
$28 \text{ mm} > \emptyset > 16$ mm of the inhibition zone	Moderately inhibitory
$16 \text{ mm} > \emptyset > 10$ mm of the inhibition zone	Slightly inhibitory
$\emptyset < 10$ mm zone of inhibition	Non- inhibitory

2.6. Assessment of antimicrobial activity by dilution method

The minimal inhibitory concentration (MIC) of the essential oil was determined by a modified broth dilution method. The essential oil was diluted to give five different concentrations (12.08, 6.04, 3.02, 1.51 to 0.76 mg/mL for *Lippia javanica* and 13.14, 6.57, 3.28, 1.62 to 0.82 mg/mL for *Lantana camara*) in the nutrient broth. Twin80, 0.01% was added to the medium to allow the solubility of essential oils. Using a standard micropipette, 0.05 mL of the 18 hours old bacterial broth (10^6 CFU/mL) culture was introduced into each of the test tubes with different concentrations

of essential oil. A set of tubes containing only the growth medium plus each of the test bacteria was set up separately to serve as controls. All tubes were incubated at 27 ± 2 °C for 30 h. The MIC was the lowest concentration of essential oil that prevented bacterial growth. The same test was repeated with the antibiotic (gentamicin) and Germicide to serve as a positive control [22]. Minimum bactericidal concentration (MBC) was determined by seeding the inoculum of each test tube in Petri dishes containing the same culture medium and incubated for 24 h at 30 °C [28]. The activities of the essential oil can thus be divided into 4 classes according to their MIC (Table 2).

Table 2. Classification of essential oils according to their minimal inhibitory concentration (MIC) [29].

MIC	Classification
MIC less than 0.1 mg/mL	Very strongly inhibitory
MIC between 0.1 - 0.5 mg/mL	Highly inhibitory
MIC between 0.6 – 1.5 mg/mL	Moderately inhibitory
MIC greater than or equal to 1.6 mg/mL	Low inhibitory

3. Results

3.1. Extractions of essential oils

The fresh aerial plant material of each sample (1000-1200 g) was subjected to hydrodistillation using the Clevenger

apparatus for 6 h. The essential oil was transferred into a stoppered tube, dried over anhydrous Na_2SO_4 and stored in a refrigerator at 4°C . Each essential oil was weighed to determine the extraction yield (Table 3; Fig. 2).

Table 3. Volume, density and yield of *Lippia javanica* and *Lantana camara* essential oils extracted

Species	Vegetable material (in g)	Oil volume (in mL)	Density	Yield (%)
<i>Lippia javanica</i>	1000	2.7	0.806	0.21 ± 0.024
<i>Lantana camara</i>	1200	1.5	0.876	0.11 ± 0.014

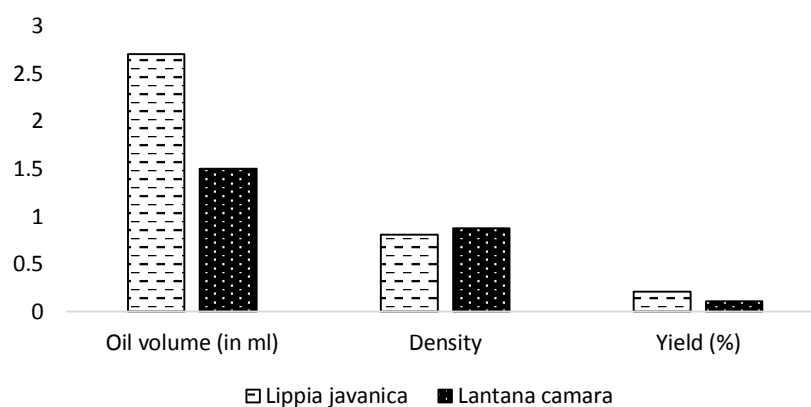


Figure 2. Volume, density, and extraction yield of of *Lippia javanica* and *Lantana camara* essential oils

From 1000 g of fresh leaves of *Lippia javanica* (Table 3; Fig. 2), 2.7 mL of essential oils was obtained. This volume represents an extraction yield of $0.21 \pm 0.024\%$. On the other hand, from 1200g of fresh *Lantana camara* leaves, 1.5 mL of essential oil was obtained, representing an extraction yield of $0.11 \pm 0.014\%$. Besides, the essential oil of *Lantana camara* was denser ($d=0.876$) than that of *Lippia javanica* ($d=0.806$).

3.2. Assessment of antimicrobial activity by dilution method

The antibacterial and antifungal activities of essential oils of *Lippia javanica* and *Lantana camara*, carried out

by the dilution method, were evaluated against Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*), Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pneumoniae*) and a fungus (*Candida albicans*). Moreover, the Germicide, a commercial antibacterial product, was used as a positive control. For each active product, the MIC, the MBC and the MBC/MIC ratio were determined to identify the bactericidal effect when the MBC/MIC ratio was equal to 1 or 2 and the bacteriostatic effect when the MBC/MIC ratio was superior to 2 [29]. The results obtained are shown in Table 4 and Figure 3 below.

Table 4. Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC) of *Lippia javanica* and *Lantana camara* essential oils tested and Germicide.

Microbial strain	<i>Lippia javanica</i>			<i>Lantana camara</i>			Germicide		
	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
<i>Escherichia coli</i>	1.5	3.02	2 ^a	1.64	6.57	4 ^b	7.08	0	0 ^c
<i>Klebsiella pneumoniae</i>	0.76	6.04	8 ^b	1.64	3.28	2 ^a	7.08	0	0 ^c
<i>Staphylococcus aureus</i>	1.50	3.02	2 ^a	3.28	6.57	2 ^a	14.15	0	0 ^c
<i>Pseudomonas aeruginosa</i>	1.50	3.02	2 ^a	3.28	6.57	2 ^a	1.77	0	0 ^c
<i>Streptococcus pneumoniae</i>	0.76	6.04	8 ^b	6.57	3.28	0.5 ^a	3.54	0	0 ^c
<i>Candida albicans</i>	3.02	0	0 ^c	3.28	0	0 ^c	7.08	0	0 ^c

Where: Bactericidal Effect (a), Bacteriostatic Effect (b), Undetermined Effect (c).

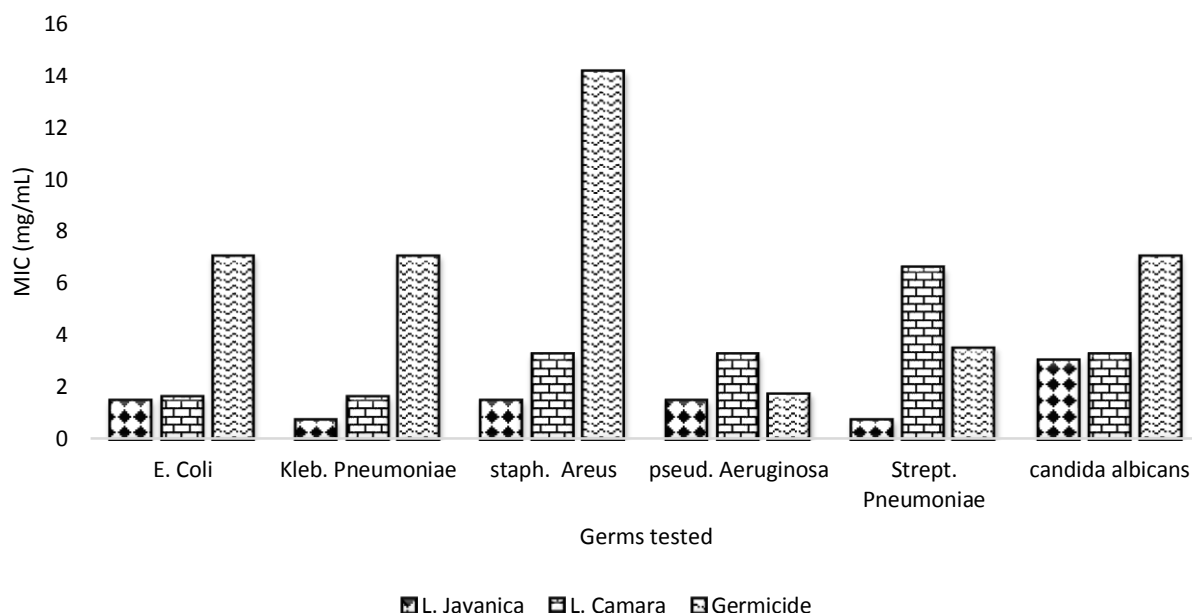


Figure 3. Comparison of of *Lippia javanica* and *Lantana camara* essential oils Minimal inhibitory concentration (MIC)

From the Table 5 and the figure 4, it appears that antibacterial and antifungal activities of the positive control, Germicidal, were completely

undetermined. Indeed, this reference product showed minimal inhibitory concentration (MIC) varying between 1.77 mg/mL on *Pseudomonas aeruginosa*

and 14.15 mg/mL on *Staphylococcus aureus*. Its antimicrobial activity is very low compared with the essential oils studied.

Moreover, the essential oil of *Lippia javanica* showed a bactericidal effect on *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* with a MIC of 1.50 mg/mL and an MBC of 3.02 mg/mL while it showed a bacteriostatic effect on *Klebsiella pneumoniae* and *Streptococcus pneumoniae* with a MIC of 0.76 mg/mL and an MBC of 6.04 mg/mL. The effect of this oil has not been determined on *Candida albicans* (MIC: 3.02 mg/mL; MBC: 0 mg/mL).

The *Lantana camara* essential oil showed a bactericidal effect on all the germs tested except on *Escherichia coli* (MIC: 1.64 mg/mL; MBC: 6.57 mg/mL) and on *Candida albicans* (MIC: 3.28 mg/mL; MBC: 0 mg/mL) on which the

effect was bacteriostatic and indeterminate, respectively.

3.4. Assessment of antimicrobial by disc diffusion method

The disc diffusion method (Table 5; Fig. 4) was also used to evaluate the antimicrobial and antifungal activities of essential oils of *Lippia javanica* and *Lantana camara*. Germicide and gentamicin were also used as the positive control. Each disc was soaked with 5-10-15 μ L of essential oil and/or Germicide. Gentamicin was used too at the concentration of 15 μ g/mL. After incubation of Petri dishes at 28-37 °C for 24 h, the inhibition zone (in mm) was measured. Moreover, according to the classification of the inhibition zone of essential oil [23, 24] (Table 1), the inhibitory power of each active substance was determined. All findings are shown in Table 5 and Fig. 4 below.

Table 5. Inhibition zone (in mm) of products tested during the assessment of antimicrobial by disc diffusion method

Microbial strain	<i>Lippia javanica</i>			<i>Lantana camara</i>			Germicide			Gentamicin 15 μ g/mL
	5 μ L	10 μ L	15 μ L	5 μ L	10 μ L	15 μ L	5 μ L	10 μ L	15 μ L	
<i>Escherichia coli</i>	5	11	19	3	6	10	2	5	8	20
<i>Klebsiella pneumoniae</i>	7	15	24	2	5	9	3	6	9	14
<i>Staphylococcus aureus</i>	0	0	0	0	0	0	0	0	0	27
<i>Pseudomonas aeruginosa</i>	6	15	21	2	5	9	2	5	8	19
<i>Streptococcus pneumoniae</i>	4	9	13	0	0	0	0	0	0	0
<i>Candida albicans</i>	4	7	12	0	0	0	0	0	0	0

With the data in table 5 above, it was draws the graph in Fig. 4 below.

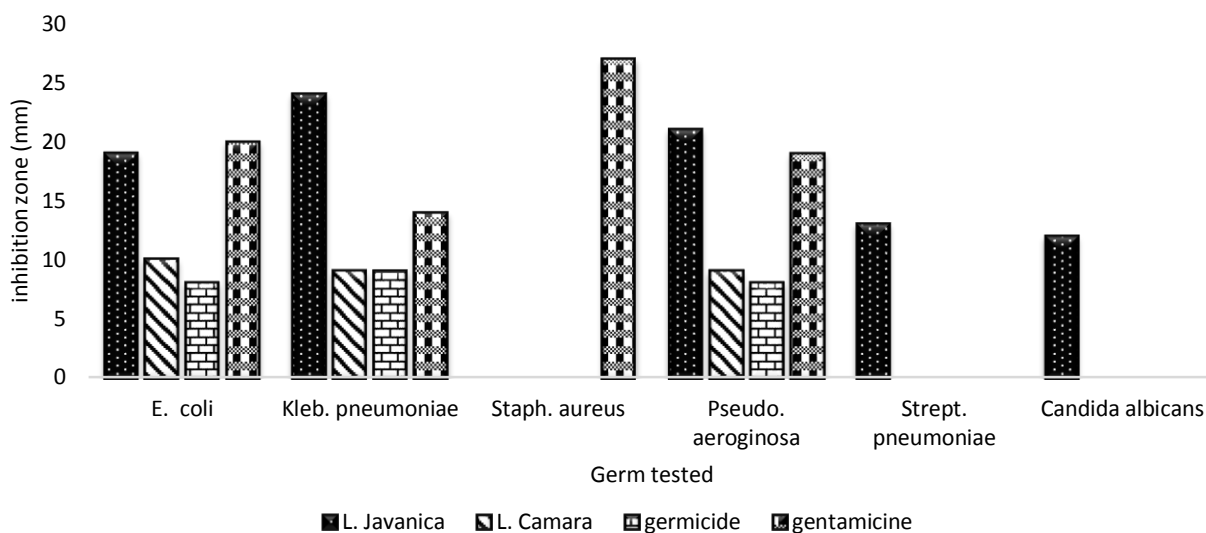


Figure 4. Evolution of inhibition zone of products tested (in mm)

It was found that *Staphylococcus aureus* (Table 5; Fig. 4) was the most sensitive to gentamicin, because of the large inhibition zone (27 mm) followed by *Escherichia coli* (20 mm), *Pseudomonas aeruginosa* (19 mm), and *K. pneumoniae* (14 mm). Moreover, *Streptococcus pneumoniae* and *Candida albicans* were found to be resistant to gentamicin with zero as an inhibition zone. Germicide, a second positive control, was inactive on *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and *Candida albicans* in the range of concentration used. Also, a weak antimicrobial activity was observed on *Klebsiella pneumoniae* (3 mm at 5 μ L, 6 mm at 10 μ L and 9 mm at 15 μ L), followed by *Escherichia coli* and *Pseudomonas aeruginosa* (2 mm at 5 μ L, 5 mm at 10 μ L and 8 mm at 15 μ L). By comparing the results of the two positive controls, gentamicin showed the greatest antibacterial activity compared with Germicide but both were inactive on *Candida albicans*.

The same tests done with essential oils show that *K. pneumoniae* was the most

sensitive germ to *Lippia javanica* essential oil with an inhibition zone which has evolved from 7 mm (5 μ L), 15 mm (10 μ L), and then to 24 mm (15 μ L). It is followed by *Pseudomonas aeruginosa* (21 mm at 15 μ L), *Escherichia coli* (19 mm at 15 μ L), and *Streptococcus pneumoniae* (13 mm at 15 μ L). *Lantana camara* essential oil was active on *Escherichia coli*, which is the most sensitive germ strains to this essential oil; its inhibition zone increased from 3 mm (5 μ L), 6 mm (10 μ L) to 10 mm (15 μ L), followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (9 mm at 15 μ L). While evaluating the antifungal activity of essential oils, it was observed that *Lantana camara* was inactive on *Candida albicans*, contrary to *Lippia javanica* essential oil that showed a slightly inhibitory effect (12 mm at 15 μ L) on *Candida albicans*.

The comparison of results obtained (15 μ L of Essential oil) by the dilution method and diffusion method shows some similarities of antimicrobial activity of essential oils obtained by the two methods (Table 6).

Table 6. Comparison of the results obtained by diffusion and dilution methods

Microbial strain	<i>Lippia javanica</i>		<i>Lantana camara</i>		Germicide	
	MIC (mg/mL)	IZ (mm)	MIC (mg/mL)	IZ (mm)	MIC (mg/mL)	IZ (mm)
	Dilution	Disk	Dilution	Disk	Dilution	Disk
<i>Escherichia coli</i>	1.50	19	1.64	10	7.08	8
<i>Klebsiella pneumoniae</i>	0.76	24	1.64	9	7.08	9
<i>Staphylococcus aureus</i>	1.50	0	3.28	0	14.15	0
<i>Pseudomonas aeruginosa</i>	1.50	21	3.28	9	1.77	8
<i>Streptococcus pneumoniae</i>	0.76	13	6.57	0	3.54	0
<i>Candida albicans</i>	3.02	12	3.28	0	7.08	0

IZ: Inhibition zone

While comparing results obtained with 15 μ L the essential oil (Table 6), it appears that the *Lippia javanica* essential oil showed moderately inhibitory activity on *Klebsiella pneumoniae* (24 mm; 0.76 mg/mL), *Pseudomonas aeruginosa* (21 mm; 1.50 mg/mL) and *Escherichia coli* (19 mm; 1.50 mg/mL). It has a slightly inhibitory activity on *Streptococcus pneumoniae* (13 mm, 1.50 mg/mL) and *Candida albicans* (12 mm, 3.02 mg/mL). This oil was inactive on *Staphylococcus aureus*. Moreover, the *Lantana camara* essential oil showed a slightly inhibitory activity only on *Escherichia coli* (10 mm, 1.64 mg/mL) and it was inactive on *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Candida albicans*.

The *Lippia javanica* essential oil showed a moderate inhibitory activity on *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* with two methods used but its activity was moderate on *Staphylococcus aureus* by the dilution method, and it was inactive on the same germ by the disc diffusion method. Moreover, this essential oil showed slightly inhibitory activity on *Streptococcus pneumoniae* and *Candida albicans* by disc diffusion method but it

presented respectively a moderate activity against *Streptococcus pneumoniae* and was inactive against *Candida albicans* by the dilution method.

Therefore, the *Lantana camara* essential oil and Germicide were inactive on all strains tested by both methods except its activity on *Escherichia coli* which was slightly by the disc diffusion method.

4. Discussion

Several works have been conducted towards the extraction of essential oil from *Lantana camara*. Deena and Thoppil [30] extracted the essential oil with a yield of 0.23% from *Lantana camara* collected in, Calicut (India). This yield is different from that obtained in the present study (0.11%). It appears that the extraction yield of this study (Table 3) is in agreement with the extraction yield of 0.1% obtained by Tesch *et al.* on *Lantana camara* collected from Táchira State (Venezuela)[31] and the yield 0.12% obtained by Costa *et al.* [32]. It is also close to that obtained by hydrodistillation (0.2%) of the air-dried leaves of *Lantana camara* from the Botanical Garden of the University of Ibadan (Nigeria). Besides, in this study, the essential oil of *Lippia*

javanica was obtained with a yield of 0.21%. In contrast, Chagonda *et al.* extracted the essential oil of *Lippia javanica* collected from two sites near Bulawayo, Western Zimbabwe with the yield ranging between 1.1 and 1.4% [33, 34]. The differences in essential oil content in the two species can be justified by seasonal variations, developmental stage of collected plant material, methods of harvest, processing of plant materials and extraction methods, and environmental conditions [33, 34].

MIC values (Table 4) were interpreted using the classification adapted by Aligiannis [35] and Duarte *et al.* [36], showing that the essential oil with MIC values greater than 0.5 mg/mL is highly inhibitory, and those with MIC values greater than 1.6 mg/mL are weakly inhibitory [29]. Gibbons [37] and Rios & Recio [38], as proposed by Van Vuuren [39], interpreted that any natural product with MIC values below 1.0 mg/mL has a remarkable antimicrobial activity and if the MIC is less than 0.1 mg/mL, it is very interesting. Van Vuuren [39] proposed, after an extensive review of essential oil, the essential oil with a MIC value less than or equal to 2 mg/mL could be considered to have interesting biological activity. In agreement with those classifications (Table 2), it appears that the *Lippia javanica* essential oil showed a moderate inhibitory activity on *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* but its activity was moderate on *Staphylococcus aureus*. Moreover, this essential oil showed slightly inhibitory activity on *Streptococcus pneumoniae* and *Candida albicans* by disc diffusion method but it presented respectively a moderate activity against *Streptococcus pneumoniae* and no activity against *Candida albicans*. Therewith, for the *Lantana camara* essential oil, its activity on *Escherichia coli* was slightly active by the disc

diffusion method but with the dilution method, it was inactive.

Costa *et al.* [32] showed that *Lantana camara* essential oil exhibited inhibitory activity on *Escherichia coli* (MIC: 512 µg/mL) and *Staphylococcus aureus* (MIC: 256 µg/mL). Another study made by the disc diffusion method showed that the *Lantana camara* essential oil exhibited significant antibacterial activity against *Escherichia coli* (10.9 mm) and *Staphylococcus aureus* (12.2 mm) but a weak inhibitory power on *Klebsiella pneumoniae* (6.3 mm) and *Pseudomonas aeruginosa* (8.5 mm) using discs soaked with 10 µL of essential oil [14]. This oil also showed an antistaphylococcus *aureus* activity with an MBC of 200 µg/mL in the study done by Kurade *et al.* [40] and a MIC of 400 µg/mL in that of Tesch *et al.* [31] while it has been inactive on *Escherichia coli* [31, 40]. Besides, the essential oil of *Lippia javanica* has shown inhibitory activity with the strongest bacteriostatic effect observed for *Klebsiella pneumoniae* [10]. This *Lippia javanica* essential oil exhibited an inhibition power against *Escherichia coli* (16 mm) and *Staphylococcus aureus* (18 mm) [41].

The antifungal activity of *L. camara* essential oil on *Candida albicans* was also reported with an inhibition zone of 14 mm by the diffusion method and a MIC of 10 mg/l for the dilution method [42]. This could be justified by the high concentration used in this study. Indeed, in the method of dilution, *Lantana camara* essential oil in this study revealed a low MIC compared to the MIC found by Sonibare and Effiong [42] whereas any activity was revealed for this oil using the disk method on *Candida albicans* in the present study.

Manenzhe *et al.* [41] found that *Lippia javanica* essential oil had a visible antimicrobial activity at 1% dilution of this essential oil on *Escherichia coli* and *S. aureus*. They also found that *Lippia*

javanica essential oil had a remarkable activity on *Candida albicans*. Also, *Lippia javanica* essential oil demonstrated significant bacteriostatic activity against *K. pneumoniae* according to the study conducted by Viljoen *et al.* [15]. These results are in agreement with the results obtained in the present study, which clearly show that *Lippia javanica* essential oil has very remarkable antimicrobial effects on *Escherichia coli*, *S. aureus*, and *Klebsiella pneumoniae*.

The essential oils and Germicide tested gave high MICs on *Candida albicans* (3.02 mg/mL for the *Lippia javanica* essential oil, 3.29 mg/mL for the *Lantana camara* essential oil, and 7.08 mg/mL for the Germicide. After the analysis of results, it was found that the Germicide tested simply inhibited the growth of the germs without having a lethal effect. It meant that the Germicide should only be used as a concentrated product to kill the germs, while essential oils even diluted can kill these germs, except *Candida albicans* for essential oils tested and *Streptococcus pneumoniae* for *Lantana camara* in the concentration range studied.

The *in vitro* evidence indicates that essential oils can act as antibacterial agents against a wide spectrum of pathogenic bacterial strains. Thus, the essential oils could possibly be used as an alternative to antibiotics [5]. Thus, when comparing the MBC/MIC ratio (Table 4), it allows to define if the natural substance test has the bactericidal effect (MBC/MIC = 1 or 2) or the bacteriostatic effect (MBC/MIC > 2) [43]. Indeed, the *Lippia javanica* essential oil exhibits a bactericidal effect on *Escherichia coli*, *S. aureus* and a bacteriostatic effect on *Klebsiella pneumoniae* and *Streptococcus pneumoniae*. However, the *L. camara* essential oil shows a bactericidal effect on all strains tested except *Escherichia coli* but the Germicide effect, on the different strains, was undefined.

However, comparing the efficacy of essential oils through different publications remains difficult to establish. This difficulty resides in the experimental parameters, in particular, the method used to evaluate the antimicrobial activity, the choice and the physiological conditions of the microorganisms, the exposure period of the microorganism to the essential oil, the dose and the emulsifier used. These parameters are different from one study to another. To these, the phytochemical divergence of the same species should be added, which is generally justified by the fact that the chemical composition of a plant species depends on the harvest, the nature of the soil and all other physical and biological characteristics of the ecosystem [44, 45].

5. Conclusion

This study shows that *Lippia javanica* essential oil showed that it had antifungal activity on *Candida albicans*, and *Lantana camara* essential oil has a slight antibacterial activity on *Escherichia coli*, *K. pneumoniae* and *Pseudomonas aeruginosa* compared with the positive controls used.

The essential oils studied show that they have a potential antibacterial and antifungal, that is why the next step will be oriented to the tests of the oils studied on the microbiological sanitation of the air and the assessment of the bio-insecticidal effect.

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Authors' contributions

Mbayo Marsi K and Kalonda ME designed this study, obtained and analyzed the data. Muhune KS and Wa

Ilunga NE supervised the collection of samples and the identification of plant species studied. Mulamba MJ, Lukusa KT, Muyumba NW, Mbayo MJ and Maloba MJ proceeded to the data quality control and the manuscript drafting. Misenga TA and Derek Ndinteh T revised the final version and translated the text. Topwe MMM, Lumbu S-JB supervised this study and corrected the manuscript.

Consent for publications

Given their contribution, all authors agree to have read the manuscript and authorize the publication of the final version of the manuscript

Conflict declaration

The authors declare that there is no conflict.

Conflict of interest

None of the authors have any conflict of interest to declare.

Availability of data and material

Data are available on request from the authors.

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No humans or animals were used in the present research.

References

1. Pibiri M-C. (2006). Assainissement microbiologique de l'air et des systèmes de ventilation au moyen d'huiles essentielles: *Lausanne, EPFL*. 177 Pages, <https://doi.org/10.5075/epfl-thesis-3311>
2. Khodadadi S, Mahdinezhad N, Fazeli-Nasab B, Heidari M J, Fakheri B, Miri A. (2021). Investigating the Possibility of Green Synthesis of Silver Nanoparticles Using *Vaccinium arctostaphylos* Extract and Evaluating Its Antibacterial Properties. *BioMed research international*, 2021: Article ID: 5572252. <https://doi.org/10.1155/2021/5572252>
3. Benabdelkader T. (2012). Biodiversité, bioactivité et biosynthèse des composés terpéniques volatils des lavandes ailées, *Lavandula stoechas* sensu lato, un complexe d'espèces méditerranéennes d'intérêt pharmacologique. Université Jean Monnet-Saint-Etienne; Ecole normale supérieure de Kouba (Alger).
4. Fazeli-Nasab B. (2021). Biological Evaluation of Coronaviruses and the Study of Molecular Docking, Linalool, and Thymol as orf1ab Protein Inhibitors and the Role of SARS-CoV-2 Virus in Bioterrorism. [Research]. *journal of ilam university of medical sciences*, 28(6): 77-96. <https://doi.org/10.29252/sjimu.28.6.77>
5. Edris A E. (2007). Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 21(4): 308-323. <https://doi.org/10.1002/ptr.2072>
6. Bakkali F, Averbeck S, Averbeck D, Idaomar M. (2008). Biological effects of essential oils—a review. *Food and Chemical Toxicology*, 46(2): 446-475. <https://doi.org/10.1016/j.fct.2007.09.106>
7. Fazeli-Nasab B, Mousavi S R. (2019). Antibacterial activities of *Ephedra sinica* herb extract on standard and clinical strains of *Pseudomonas aeruginosa*. *Journal of Medical Bacteriology*, 8(3, 4): 40-48.

8. Fazeli-nasab B, Moshtaghi N, Forouzandeh M. (2019). Effect of Solvent Extraction on Phenol, Flavonoids and Antioxidant Activity of some Iranian Native Herbs. *Scientific Journal of Ilam University of Medical Sciences*, 27(3): 14-26 <https://doi.org/10.29252/sjimu.27.3.14>
9. Belaiche P. (1979). *Traité de phytothérapie et d'aromathérapie: Les maladies infectieuses: Maloïne, Paris.*
10. Valizadeh M, Beigomi M, Fazeli-Nasab B. (2020). Antibacterial and Anti biofilm effects of ethanol and acetone leaf extract of *Momordica charantia* and *Tecomella undulata* against *Acinetobacter baumannii*. *International Journal of Advanced Biological and Biomedical Research*, 8(4): 403-418. <https://doi.org/10.33945/sami/ijabbr.2020.4.6>
11. Boukhatem M N, Hamaidi M S, Saidi F, Hakim Y. (2010). Extraction, composition et propriétés physico-chimiques de l'huile essentielle du Géranium Rosat (*Pelargonium graveolens* L.) cultivé dans la plaine de Mitidja (Algérie). *Nature & Technology*(3): 37.
12. Endris A, Asfaw N, Bisrat D. (2016). Chemical composition, antimicrobial and antioxidant activities of the essential oil of *Lippia javanica* leaves from Ethiopia. *Journal of Essential Oil Research*, 28(3): 221-226. <https://doi.org/10.1080/10412905.2015.1108880>
13. Bardaweel S K, Bakchiche B, ALSalamat H A, Rezzoug M, Gherib A, Flamini G. (2018). Chemical composition, antioxidant, antimicrobial and Antiproliferative activities of essential oil of *Mentha spicata* L.(Lamiaceae) from Algerian Saharan atlas. *BMC Complementary and Alternative Medicine*, 18(1): 1-7. <https://doi.org/10.1186/s12906-018-2274-x>
14. Saikia A K, Sahoo R K. (2011). Chemical composition and antibacterial activity of essential oil of *Lantana camara* L. *Middle-East Journal of Scientific Research*, 8(3): 599-602.
15. Viljoen A, Subramoney S v, Van Vuuren S, Başer K, Demirci B. (2005). The composition, geographical variation and antimicrobial activity of *Lippia javanica* (Verbenaceae) leaf essential oils. *Journal of Ethnopharmacology*, 96(1-2): 271-277. <https://doi.org/10.1016/j.jep.2004.09.017>
16. Pascual M, Carretero M, Slowing K, Villar A. (2002). Simplified screening by TLC of plant drugs. *Pharmaceutical Biology*, 40(2): 139-143. <https://doi.org/10.1076/phbi.40.2.13.95849>
17. York T, De Wet H, Van Vuuren S. (2011). Plants used for treating respiratory infections in rural Maputaland, KwaZulu-Natal, South Africa. *Journal of Ethnopharmacology*, 135(3): 696-710. <https://doi.org/10.1016/j.jep.2011.03.072>
18. Diomande L B, Kanko C, Tia E V, Kone B, Yao-Kouamé A. (2014). Occurrence et composition chimique de l'huile essentielle des feuilles de *Lippia multiflora* M.(thé de savane) selon le pH, les teneurs en Carbone, en Azote et Phosphore du sol en zones de savane guinéenne en Côte d'Ivoire. *Afrique Science: Revue Internationale des Sciences et Technologie*, 10(4): 93-108.
19. Burkill H M. (1995). The useful plants of west tropical Africa, Vols. 1-3. *The useful plants of west tropical Africa, Vols. 1-3.*(2. ed.).
20. Kalita S, Kumar G, Karthik L, Rao K V B. (2012). A Review on Medicinal Properties of *Lantana camara* Linn. *Research Journal of Pharmacy and Technology*, 5(6): 711.
21. Benayache F. (2013). Etude phytochimique et biologique de

- l'espèce *Thymus numidicus* Poiret: *Mémoire de master en chimie organique, Université Constantine, Algérie.*
22. Naidoo Y, Sadashiva C, Kasim N, Nicholas A, Naidoo G. (2014). Chemical composition and antimicrobial activity of the essential oil of *Ocimum obovatum* E. Mey. Ex Benth.(Lamiaceae). *Journal of Essential Oil Bearing Plants*, 17(1): 142-147. <https://doi.org/10.1080/0972060X.2014.884782>
 23. Naidoo Y, Sadashiva C, Naidoo G, Raghu K. (2016). Antibacterial, antioxidant and phytochemical properties of the ethanolic extract of *Ocimum obovatum* E. Mey. ex Benth: *NISCAIR-CSIR, India*. 356 Pages,
 24. Sadgrove N, Jones G. (2015). A contemporary introduction to essential oils: chemistry, bioactivity and prospects for Australian agriculture. *Agriculture*, 5(1): 48-102. <https://doi.org/10.3390/agriculture5010048>
 25. Brahim M A S, Fadli M, Hassani L, Boulay B, Markouk M, Bekkouche K, Abbad A, Ali M A, Larhsini M. (2015). *Chenopodium ambrosioides* var. *ambrosioides* used in Moroccan traditional medicine can enhance the antimicrobial activity of conventional antibiotics. *Industrial Crops and products*, 71: 37-43. <https://doi.org/10.1016/j.indcrop.2015.03.067>
 26. Aboul Ela M, El-Shaer N, Ghanem N. (1996). Antimicrobial evaluation and chromatographic analysis of some essential and fixed oils. *Pharmazie*, 51(12): 993-994.
 27. Meena M. (1994). Antimicrobial activity of essential oils from spices. *J. Food Sci. Technol.*, 31: 68-70. NII Article ID (NAID): 80008001794
 28. El Kalamouni C. (2010). Caractérisations chimiques et biologiques d'extraits de plantes aromatiques oubliées de Midi-Pyrénées. (PhD), Institut National Polytechnique de Toulouse.
 29. Suliman S. (2011). Antimicrobial interaction of *Artemisia afra* used in African traditional medicine. (M.Sc.), Faculty of Health Sciences, University of the Witwatersrand, Johannesburg.
 30. Deena M, Thoppil J. (2000). Antimicrobial activity of the essential oil of *Lantana camara*. *Fitoterapia*, 71(4): 453-455. [https://doi.org/10.1016/S0367-326X\(00\)00140-4](https://doi.org/10.1016/S0367-326X(00)00140-4)
 31. Tesch N R, Mora F, Rojas L, Díaz T, Velasco J, Yáñez C, Rios N, Carmona J, Pasquale S. (2011). Chemical composition and antibacterial activity of the essential oil of *Lantana camara* var. *moritziana*. *Natural Product Communications*, 6(7): 1934578X1100600727. <https://doi.org/10.1177/1934578X1100600727>
 32. Sousa E O, Silva N F, Rodrigues F F, Campos A R, Lima S G, Costa J G M. (2010). Chemical composition and resistance-modifying effect of the essential oil of *Lantana camara* Linn. *Pharmacognosy magazine*, 6(22): 79. PMID: PMC2900066; PMID: 20668570; <https://doi.org/10.4103/0973-1296.62890>
 33. Chagonda L S, Makanda C D, Chalchat J-C. (2000). Essential oils of wild and cultivated *Lippia javanica* (Spreng) and *L. oatesii* (Rolfe) from Zimbabwe. *Journal of Essential Oil Research*, 12(1): 1-6. <https://doi.org/10.1080/10412905.2000.9712027>
 34. Chagonda L S, Chalchat J-C. (2015). Essential oil composition of *Lippia javanica* (Burm. f.) spreng chemotype from Western Zimbabwe. *Journal of Essential Oil Bearing Plants*, 18(2): 482-485.

- <https://doi.org/10.1080/0972060X.2014.1001140>
35. Aligiannis N, Kalpoutzakis E, Mitaku S, Chinou I B. (2001). Composition and antimicrobial activity of the essential oils of two *Origanum* species. *Journal of agricultural and food chemistry*, 49(9): 4168-4170. <https://doi.org/10.1021/jf001494m>
 36. Duarte M C T, Figueira G M, Sartoratto A, Rehder V L G, Delarmelina C. (2005). Anti-Candida activity of Brazilian medicinal plants. *Journal of Ethnopharmacology*, 97(2): 305-311. <https://doi.org/10.1016/j.jep.2004.11.016>
 37. Gibbons S. (2004). Anti-staphylococcal plant natural products. *Natural product reports*, 21(2): 263-277. <https://doi.org/10.1039/B212695H>
 38. Rios J-L, Recio M C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology*, 100(1-2): 80-84. <https://doi.org/10.1016/j.jep.2005.04.025>
 39. Van Vuuren S. (2008). Antimicrobial activity of South African medicinal plants. *Journal of Ethnopharmacology*, 119(3): 462-472. <https://doi.org/10.1016/j.jep.2008.05.038>
 40. Kurade N P, Jaitak V, Kaul V K, Sharma O P. (2010). Chemical composition and antibacterial activity of essential oils of *Lantana camara*, *Ageratum houstonianum* and *Eupatorium adenophorum*. *Pharmaceutical Biology*, 48(5): 539-544. <https://doi.org/10.3109/13880200903193336>
 41. Manenzhe N J, Potgieter N, van Ree T. (2004). Composition and antimicrobial activities of volatile components of *Lippia javanica*. *Phytochemistry*, 65(16): 2333-2336. <https://doi.org/10.1016/j.phytochem.2004.07.020>
 42. Sonibare O O, Effiong I. (2008). Antibacterial activity and cytotoxicity of essential oil of *Lantana camara* L. leaves from Nigeria. *African Journal of Biotechnology*, 7(15): 2618-2620.
 43. Mbayo M K, Kalonda E M, Tshisand P T, Tatchoua O, Kamulete S, Mbayo G K, Kihuya E N, Kahumba J B, Lumbu J-B S. (2015). Criblage chimique de quelques champignons du Katanga (RDC) et évaluation de leur activité biologique [Chemical Screening of some mushrooms of Katanga (DRC) and their biological activities evaluation]. *International Journal of Innovation and Applied Studies*, 10(1): 435-449.
 44. Ameen O, Usman L, Oganija F, Hamid A, Muhammed N, Zubair M, Adebayo S. (2011). Chemical composition of leaf essential oil of *Annona senegalensis* Pers.(Annonaceae) growing in North Central Nigeria. *International Journal of Biological and Chemical Sciences*, 5(1). <https://doi.org/10.4314/ijbcs.v5i1.68117>
 45. Mbayo M, Kalonda E, Muya R, Tshisand P, Kanangila A, Maseho F, Kihuya E, Bakari S, Kahumba J, Lumbu J. (2016). Test d'activité antimicrobienne et étude chimique préliminaire de quelques Euphorbiaceae du Katanga méridional (RDC). *Phytothérapie*: 1-13. <https://doi.org/10.1007/s10298-016-1060-5>

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