ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL FROM *LANTANA CAMARA*

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Abstract

***Lantana camara* is a shrub belonging to the Verbenaceae family. This shrub is common in both tropical and warm regions worldwide. It has been used in outdoor landscapes, as hedges and fencelines because of its thick thorny nature and its bright flowers. Crude extracts from *L. camara* have commonly been used ethnobotanically as a herbal medicine with perceived anti-protozoal, anti-bacterial and antifungal activities. However, the antimicrobial activity of essential oil from *L. camara* is not well documented. The objective of this study was to determine the bioactivity of essential oils from *L. camara* against *Ralstonia solanacearum* and *Furarium oxysporium* that cause loses in tomatoes through wilts and also against *Escherichia coli* and *Staphylococuss aureus* that are common human pathogens responsible for food poisoning. Essential oil was obtained from powdered dried tender leaves through steam distillation. Anti-bacterial activity was assessed using the disk diffusion method against *R. solanacearum, E. coli* and *S. aureus.* Poison food technique was used to assess the antifungal activity against *F. oxysporium*. Essential oil from *L. camara* significantly inhibited the bacterial growth compared to antibiotics (p = 6.82E-05). There was no significant difference on the overall inhibition activity of essential oil against the tested bacteria (p = 0.63). Growth inhibition for *F. oxysporium* was relatively lower at 47.8% compared to positive antifungal controls but significantly higher than the negative control. The essential oil from *L. camara* significantly inhibited the growth of plant and human pathogenic microbes tested in-vitro. These oils possess great potential as antifungal-antibacterial agents against human and plant diseases.**

Key words: *Lantana camara,* antimicrobial activity, essential oil

INTRODUCTION

There is an emerging increase in the number of microbial strains resistant to recommended antibiotics rendering most of the antimicrobials ineffective due to the continuous evolution of pathogens to resistance (Salada et al., 2015). There is a need therefore, for new formulations of antimicrobial agents derived from natural plant products to address resistance (Salada et al., 2015).

Fusarium oxysporum is a plant pathogenic fungus that causes '*Fusarium* wilt' in more than a hundred species of plants including tomato, potato, sugarcane and cowpea (Sharma & Kumar, 2009a). Management of this pathogen is crucial as it affects a wide variety of host plants of economic value (Sharma & Kumar, 2009a). The increase in the development of resistance towards conventional fungicides and their increasing toxic effect on the environment creates a pressing need to look for unique plant protectants (Girish, 2017). The use of natural products from plants to control fungal diseases is considered a good alternative to synthetic fungicides, due to their lower negative impacts on the environment (Girish, 2017).

Ralstonia solanacearum is one of the most critical soil-borne plant pathogenic bacteria that causes bacterial wilt in tomato and many other crops in tropical and subtropical regions, leading to substantial economic losses (Vu et al., 2017). This bacterium has a broad host range of more than 50 botanical families that represent more than 200 plant species, including tomato, potato, eggplant, pepper, peanut, tobacco, banana, groundnut, olive, and ginger (Vu et al., 2017). The yield losses caused by this plant pathogen vary from 0-91% for tomato, 33-90% in potato, 10-30% in tobacco, 80-100% in banana and up to 20% in groundnut (Vu et al., 2017). Plant extracts, isolated compounds, and essential oils have commonly been used in trials to suppress bacterial wilt disease (Vu et al., 2017). To date, no full-proof solution has been identified to control this disease.

Staphylococcus aureus and *Escherichia coli* are human pathogens that cause multiple infections, including food poisoning (Ashraf et al., 2017). Food poisoning is commonly manifested as a diarrheal disease which is often triggered by toxin production or by the host's reaction to infection (Nicoline et al., 2015). Amongst other bacteria, *S. aureus* and *E.coli* have been associated with food of animal origin. Staphylococci are normal inhabitants of the skin and mucous membranes of both animals and humans. Strains with pathogenic potential are known to cause diseases which range from simple abscesses and mastitis to more severe toxic shock syndrome (Nicoline et al., 2015).

Escherichia coli occur as normal flora in the gastrointestinal tract of humans and animals. Pathogenic strains of *E.coli* have also been reported to cause life-threatening infections in humans worldwide (Nicoline et al., 2015). The development of drug-resistant pathogenic microorganisms is a major challenge in human and animal health (Sharma & Kumar, 2009b). Food contaminated with antibiotic-resistant bacteria is a significant threat, especially to public health as these antibiotic-resistant determinants can be transferred to other bacteria of clinical significance (Nicoline et al., 2015). This can further increase the number of food borne illnesses and the potential for treatment failure. Thus the need to continuously search for new antimicrobial compounds, especially from biological sources (Sharma & Kumar, 2009b).

Lantana camara is a shrub of the Verbenaceae family with distribution in tropical, subtropical and temperate regions (Dos Santos et al., 2015). Reports have indicated that leaves of *L. camara* have antimicrobial, fungicidal, insecticidal and nematicidal activity (Deena and Thoppil, 2000**)**. Crude extracts from *L. camara* have commonly been used ethnobotanically as a herbal medicine with perceived anti-protozoal, anti-bacterial and antifungal activities to treat various human diseases such as skin itches, leprosy, cancers, chickenpox, measles, asthma, ulcers, tumours, high blood pressure, tetanus, rheumatism (Sonibare & Effiong, 2008**).** Several authors have also studied the essential oil of *L. camara*, and their chemical composition reported to differ based on the geographical location (Semde et al., 2018).The major compounds of essential oil of *L. camara* dried leaves were reported to comprise of caryophyllene oxide (23.015%), spathulenol (13.421%), humulen-1, 2-epoxide (8.046%), β-caryophyllene (7.93%), E-nerolidol (6.933%) and α-humulene (4.925%) (Semde et al., 2018). However, little is known about the antimicrobial activity of *L. camara* essential oil in Kenya.

MATERIALS AND METHODS

Plant Sample Collection and Extraction of the Essential Oils

Natural populations of *Lantana camara* plant leaves (Fig. 1) were sampled from Jomo

Kenyatta University of Agriculture and Technology (JKUAT) botanical garden, Nairobi, Kenya. Matured leaves were picked up respectively from four directions (north, south, east, and west) obtaining as many individuals as possible. The leaves from the sampling site were harvested from the first four leaves of the stem from the top and mixed to make a composite sample. The samples were transferred to the lab within two days of collection in ventilated nylon gunny bags. The samples were washed and then air-dried immediately under room temperature (23–26 °C) in a well-ventilated room for two weeks until they turn crisp.

The protocol for essential oil extraction was adapted from Semde et al. (2018) with modifications. Two hundred grams (200g) of dried leaves were extracted by steam distillation using a Clevenger apparatus at temperatures between 80-100°C. During the steam distillation process, steam passes through the plant material. The combination of heated steam and gentle pressure causes the essential oil to be released from microscopic protective sacs. As the vapor mixture flows through a condenser and cools, it yields a separate layer of oil and of water. The essential oil rises to the top and is separated from the hydrosol using a separating funnel. The oil was collected and weighed into small amber-colored vials, labelled and stored at 4 °C until further use. The percentage yield of the oil was determined using the formula;

$$\text{Percentage Oil yield} = \frac{\text{weight of oil}}{\text{weight of powder}} * 100 \ldots (1)$$

Figure1: Leaves of *Lantana camara*

Screening for Antimicrobial Activity of Essential Oil

Screening for Anti-bacterial Activity

The anti-bacterial activity of essential oil from *L. camara* was assessed through the disk diffusion method against *R. solanacearum, E. coli* and *S. aureus* adapted from Semde et al, (2018). Stock cultures of each bacteria were prepared by inoculating a loopful of each test bacteria separately into Lauryl Bertani Broth (L.B.) and incubated at 37°C, overnight. Fifty microliters (50 µl) of the stock culture was transferred into fresh L.B. broth and incubated at 37°C for 24 hrs. The resultant suspension was then used to prepare serial dilutions for the bio-assays. One hundred microliters (100µl) of test bacteria suspension was inoculated onto Mueller Hinton agar (MHA) and spread to dry using a glass rod under sterile conditions. Sterile paper disks of 6mm in diameter were impregnated with 15µl (52.5µg) of the essential oil for treatments and 21µl (52.5µg) of Streptomycin and sterile distilled water (SDW) as positive and negative controls, respectively. The assay was done in triplicate. After air-drying, the paper disks were then placed radially on the bacteria inoculated plates at equidistance (30mm) from the center of the plate. The plates were then incubated at 37°C for 24 hrs. The activity of essential oil was determined by measuring the inhibition zone in mm.

Screening for Antifungal Activity

Antifungal activity of the essential oil was assessed against *Fusarium oxysporium* using food poison technique adapted from Choudhury et al. (2017). Forty microliters (40µl) of the essential oil was mixed with 20ml of molten potato dextrose agar (PDA) and allowed to cool. Nine millimeters (9mm) of the fungal plug from a 3-5 days old cultures was inoculated onto the poisoned media at the center of the plate. Control plates inoculated with 1mg/ml Ridomyl® (Metalaxyl) and some without essential oil ran parallel as a positive and negative control, respectively. The plates were then incubated at 25°C for five days. The percentage inhibition was determined using the formula;

$$\text{Inhibition } \% = \frac{\text{Gc} - \text{Gt}}{\text{Gc}} * 100 \ldots (2)$$

where Gc: Radial diameter of control, Gt: Radial diameter of Test

Data Analysis

The inhibition zones measured in millimeters (mm) and their means were expressed as percentage inhibition using equation 2. The experimental data was subjected to ANOVA to determine significant differences among samples.

RESULTS

The extracted essential oil from the dried leaves of *L. camara* produced a pale yellow liquid with a yield of 0.35% w/w (Fig 2). *Lantana. camara* essential oil exhibited both anti-bacterial and antifungal activities in bio-assays. Although the anti-bacterial activity of the *L. camara* essential was highest on *S. aureus* followed by *R. solanacearum* and the least was on *E. coli* (Table 1 and Fig 3) based on their zones of inhibition, there was no significant difference in the overall activity of the *L. camara* ($p=0.63$) amongst the tested bacteria. However, there was a significant difference in the anti-bacterial activity of the *L. camara* essential oil to that of standard antibiotic (p=6.82E-05). The essential oil also inhibited the growth of the test fungi *F. oxysporium* by 48.7% (Fig 4)**.**

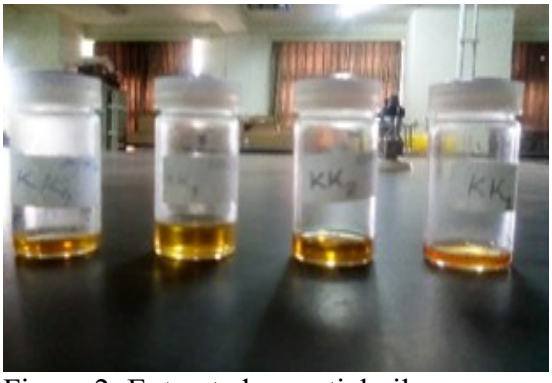

Figure 2: Extracted essential oils

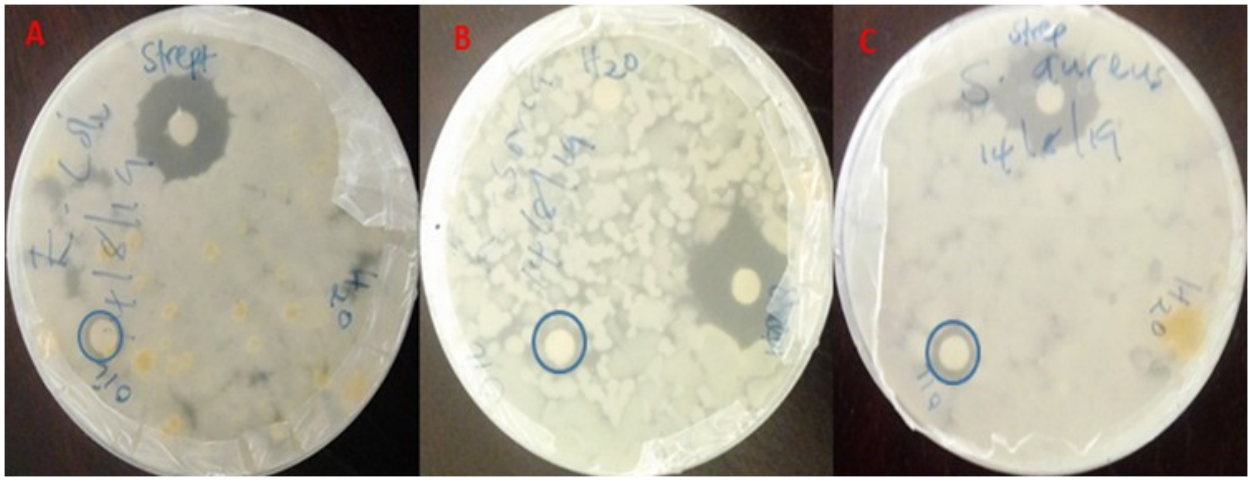

Figure 3: Anti-bacterial activity of *L. camara* essential oil. A: *E. coli, B: R. solanacearum, C: S. aureus.* Marked areas indicate zone of inhibition (mm) by the essential oil

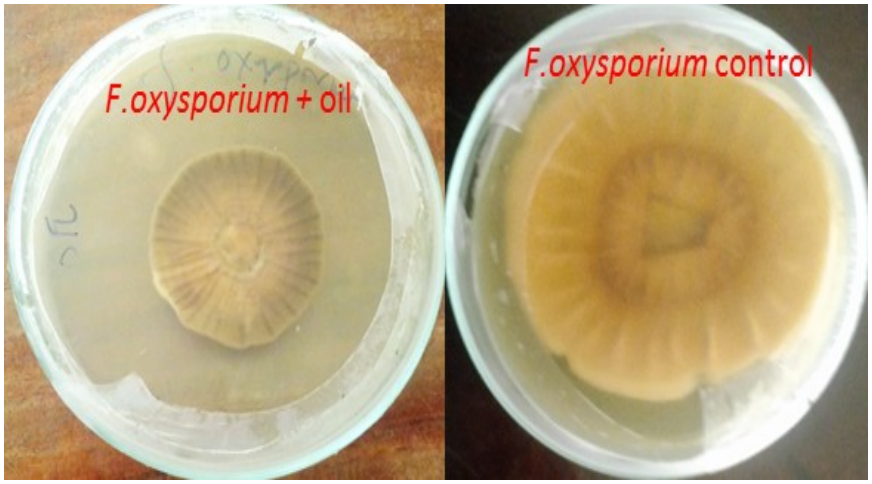

Figure 4: Antifungal activity of essential oil from *L. camara* against *F. oxysporium*

Table 1: Antimicrobial activity of the Essential Oil from *L. camara*

Microorganism	**Zone of inhibition (mm)**				**Percentage inhibition**
	Essential Oil	**Streptomycin**	**Ridomyl® Metalxyl**	**SDW Control**	
R. solanacearum	8.00	20.67	-	0	61.3
S. aureus	8.67	22.33	-	0	61.2
E. coli	7.67	20.00	-	0	61.7
F. oxysporium	44.00	-	8.00	76.00	47.8

Essential Oil (52.5ug), streptomycin concentration 52.5ug and Ridomy® (Metalaxyl) at 1mg/ml. Zone of inhibition includes the diameter of the disk, an average of three consecutive assays

DISCUSSION

To the best of our knowledge, this is the first study to describe the antimicrobial activity of essential oil from *L. camara* against important plant and human pathogens. This study showed that essential oils from *L. camara* had

significant antimicrobial activity against the test pathogens compared to the negative controls. The growth of all test pathogens was suppressed by the essential oils though at different intensities. Our results corroborate findings of Tesch et al., (2011) who reported activity of essential oil against *S. aureus.* However, Tesch et al., (2011) showed that the essential oil did not have activity against *E. coli* compared to the present study which showed activity against this bacteria. Findings from this study also corroborate those reported by Deena and Thoppil (2000) who observed that essential oil of *L. camara* had antimicrobial activity towards both Gram-positive as well as negative bacteria. However, the anti-bacterial effect of essential oils against Gram-negative bacteria was less compared to that in Gram-positive bacteria. The ability of essential oil to exhibit anti-bacterial activity is contributed by its different components whose mode of action involve several targets on the bacterial cell. Components in essential oils may degrade the cell walls, damage the cytoplasmic membrane and destroy the membrane proteins that eventually leads to leakage of cell contents. Coagulation of cytoplasm and depletion of the proton motive force is another antibiotic pathway of these essential oils (Nitin et al., 2010*).*

This present study has also shown that *L. camara* essential oil has anti-bacterial activity against *R. solanacereum* which causes bacterial wilt in tomatoes. This is the first report on the activity of essential oil from *L.camara* in bacterial wilt control. Various studies have reported effective activity of different plant metabolites such as thymol, palmarosa oil, and lemongrass oil in soils infested by *R. solanacearum* species (Vu et al., 2017).

This study has shown that essential oil from *L. camara* inhibited the growth of *F. oxysporium.* The fungi counteracted the oil by developing more pigmentation , sclerotia, concentric rings and some aerial mycelia which indicate the presence of stress factors in its growth. However, Ridomyl suppressed growth by 89% compared to 48% observed against essential oils. Girish (2017) posted similar results using a *F. oxysporium* as a test fungi where the oil inhibited the radial growth of *F. oxysporium.* As reported by Pradhanang et al., (2003) different formulated plant extracts including essential oils were shown to be effective in reducing soil populations of *F. oxysporium* and reduce *Fusarium* wilt incidence in muskmelon. The ability of essential oil to inhibit the growth of fungi could have been; as a result cell lysis at the point of contact, or the oil alterered the integrity of the cell membrane by depleting the ergosterol content **(**Girish, 2017).

CONCLUSION

The increase in the hazardous effects caused by chemical used to control plant disease, and increase in antibiotic resistance of human pathogens demands an urgent need for new alternatives. Results in this study have shown that essential oils from *L. camara* exhibit both anti-bacterial and antifungal activities. These observations and findings provide evidence that essential oil derived from *L. camara* leaves have the potentials for safe alternatives to plant and human disease control. The oil extraction process is easy and fast and can be upscaled for bulk production. Agronomically *L. camara* is considered a persistent weed on arable land, is easy to grow and a vast ecological region. Its application as a source of antimicrobial agents will be a welcomed source of livelihood in areas vastly covered by this beautiful ornamental plant usually considered a weed in many regions.

RECOMMENDATION

Most studies revealing that plant-derived products and metabolites have the potential for use as both anti-bacterial and antifungal agents

have mainly been done *in vitro. In vivo* field studies have rarely been done thus limiting information on their practical applications. It will be interesting, therefore to observe the effect of these oils in vivo setups to control fusarium and bacterial wilts which to date has no known cure. Exposure of microbes to stress is usually counteracted by the release of the compound to sanitize the stress. Some of the released compounds may be toxic to plant and human. The total effect of microbes to the biochemistry of plants and the environment needs to be considered for evaluation. The bioactive compounds in essential oil and their mode of action against microbes need to be evaluated. Such studies may lead to the development of novel antimicrobial compounds for application in human medicine and agriculture.

ACKNOWLEDGEMENT

The authors are grateful to Pwani University and Jomo Kenyata University of Agriculture and Technology for providing the laboratory space to carry out this work

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