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ANTIMICROBIAL ACTIVITY OF LEAF AND FLOWER EXTRACTS OF *LIPPIA NODIFLORA* L. (VERBENACEA)

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Abstract: Antimicrobial activities of the methanolic extracts from the leaves and flowers of *Lippia nodiflora* L. (Verbenaceae), were studied by the disk diffusion method. The extracts showed antimicrobial impact on bacteria such as *Bacillus subtilis, B. cereus, Micrococcus luteus, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, K. oxytoca* and *Esherichia coli* as well as fungi such *Aspergillus niger* and *Candida albicanse.* The results showed that increasing concentrations of extracts increased the antimicrobial activities in all of the microorganisms. Bacteria were more sensitive than fungi, and gram positive bacteria were more sensitive than gram negative ones.

Key words: antimicrobial activity, *Lippia nodiflora* L., methanolic extracts, bacteria, fungi

INTRODUCTION

Medicinal plants have been well-known natural sources of remedies for the treatment of various diseases since antiquity. According to a report by the World Health Organization (WHO), nearly 20,000 plant species are currently being used for medicinal purposes.

Over-usage of antibiotics has resulted in an increase in the resistance of bacteria against these drugs. The use of too many antibiotics can also cause numerous side effects in humans. Since some herbs have anti-microbial activity, they could be used as harmless substitute for antibiotics in the treatment of various diseases. The use of medicinal herbs in the world, contributes significantly to primary health care (Scorzoni *et al.* 2007).

The genus *Lippia* (Verbenaceae) includes approximately 200 species of herbs, shrubs, and small trees. Most of these species are traditionally utilized as remedies for some disease (Pascual *et al.* 2001). *Lippia nodiflora* L. is a perennial herb which grows in a humid environment near river banks, in tropical and subtropical regions. The aerial parts of the plant have medicinal properties and are used in many countries. The plant has also been reported as having vermifuge, antiseptic, antitussive, antipyretic and anti-inflammatory agents and finds uses in treatment of osteoarticular pains and bronchitis respiratory diseases (Bina *et al*. 2007). Phytochemical investigations on this plant have resulted in the isolation of flavone glycosides, alkaloids, essential oil, resin, stigmasterol, β-sitosterol, sugars, mono and diflavone, sulphates of neptin, jaceosidin, hispidulin and 6hydroxyluteoli (Basu *et al.* 1969; Nair *et al.* 1973; Francisco *et al*. 1987; Forestieri *et al.* 1996). Recent studies on the chemical components of this plant resulted in the finding of a new terpenoide known as lippiacian and another component named halleridone (Siddiqui *et al.* 2007).

The objective of this study was to examine the antimicrobial effect of the methanolic extract of leaves and flowers of *L. nodiflora* on some bacteria and fungi.

MATERIALS AND METHODS

Plant material

The leaves and flowers of *L. nodiflora* were collected from Khozestan, Iran, in May and June of 2009.

Preparation of extracts

Leaves and flowers of the plant were collected and in a shadowy place they were spaced apart so they could dry. Then they were ground to powder. A percolation method was used to get an extract of 50 grams of each powder with the use of 80 percent methanol. First, the powder was soaked in 80 percent methanol for one hour, and then it was put into the percolator and after 48 hours the extract (sap) was collected. Using a rotary machine set at a temperature of 40°C, the extract was concentrated and finally dried in an oven at the same temperature and its anti-bacterial characteristics were studied (Manikandan *et al.* 2009).

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Antibacterial activity assay

The disc-diffusion assay was used to determine the growth inhibition of micro-organisms by the plant extracts. The following bacteria and fungi were used: *Bacillus subtilis, B. cereus, Micrococcus luteus, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, K. oxytoca and Escherichia coli, Aspergillus niger* and *Candida albicans*.

These were maintained at 4°C on nutrient agar plates. 10 ml of Mueller Hinton Agar was poured into the petri plates and the agar was allowed to solidify. Standardized inoculum suspension (0.1 ml) was added and spread uniformly on the medium surface (Manikandan *et al.* 2009). The discs were then applied and plates were incubated at 37°C for 24 h. The inhibition zone was measured from the edge of the disc to the inner margin of the bacterial colony. Manikandan was used as a negative control, and gentamycin and nistatin were used as a positive control. The experiment was done in triplicate (Manikandan *et al.* 2009).

Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) of the plant extracts was tested in Mueller Hinton Broth by the two-fold serial dilution method. The extract concentrations used, were: 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 mg/ml. The culture tubes were incubated at 37°C for 24 h. The lowest concentration, which did not show any growth of the tested organism after microscopic evaluation, was determined as the minimum, inhibitory concentration (Manikandan *et al.* 2009).

RESULTS

The present study showed that the two extracts of *L. nodiflora* were very effective against all of micro-organisms used in this research ($p < 0.05$). Antibacterial activities were higher than fungi in both extracts. The results indicated that by increasing the concentration of extracts, the antimicrobial activities also increase (Table 1). *M. luteus, P. aeroginosa* and *K. oxytoca* showed more antibacterial activities than the other tested species. Gram positive bacteria were more sensitive than gram negative. There was no significant difference between the antimicrobial activity of the leaf and the flower extracts ($p > 0.05$) and the antimicrobial activity between the two extracts was similar. But at a concentration of 12.5% on *S. aureus*, and a 50% concentration on *C. albicanse,* there was a significant difference ($p < 0.05$) and the antibacterial activity in the flower extract was higher than the leaf extract. At a concentration of 12.5% on *M. luteus,* the antibacterial activity in the leaf extract was higher than that of the flower extract.

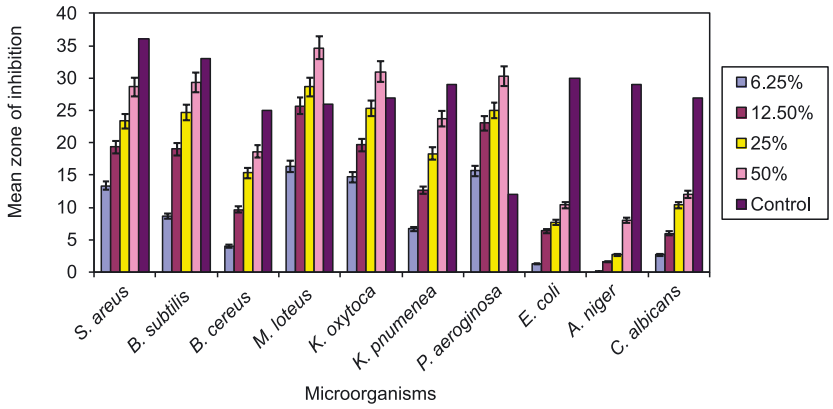

Fig. 1. Leaf methanolic extract of *L. nodiflora*

Table 1. Flower methanolic extract of *L. nodiflora*

Microorganisms	Mean zone of Inhibition [mm] ±SE							
	50%	25%	12.5%	6.25%	dimethyl sulfoxide (DMSO)	entamycin	nistatin	MIC*
Staphylococcus areus	29.33±0.33	26.33±0.88	21.66±0.88	13.00±2.51	–	37		6.25
Bacillus subtilis	29±1.00	24.66±0.88	17.33±2.90	5.00±1.73	–	36		6.25
B. cereus	19.33±0.33	14.66±1.85	9.33±0.33	4.00±2.30	–	25		6.25
Micrococcus loteus	34.00±1.15	29.66±0.33	25.33±1.76	12.33±2.96	–	28		6.25
Kelebsiella oxytocal	30.00±0.57	19.66±0.88	18.66±0.33	13.66±2.40	–	33		6.25
K. pnumonea	21.00±1.00	16.66±0.33	11.66±1.45	5.33±0.33	–	28		6.25
Pseudomonas aeroginosa	30.33±1.45	26.66±0.66	24.00±1.45	16.66±1.80	–	11		3.125
Escherichia coli	12.33±0.88	8.66±0.66	6.66±0.33	1.00±1.00	–	32		6.25
Aspergillus niger	7.00±0.57	4.00±0.57	1.33±0.66	00.00±0.00	–		38	12.5
Candida albicans	18.00±1.15	10.00±0.57	4.66±0.17	2.33±0.33	–		35	6.25

*minimum inhibitory concentrations

DISCUSSION

L. nodiflora, based on studies by many researchers, has been found to have a similar composition to other *Lippia* spp. Phytochemical investigations on this plant have resulted in the isolation of several flavone glycosides, including lippiflorin A & B, nodiflorin A & B, nodifloritin A & B, alkaloids, essential oil, resin, stigmasterol, β-sitosterol, sugars, mono and diflavonesulphates of neptin, jaceosidin, hispidulin and 6-hydroxyluteolin (Basu *et al.* 1969; Nair *et al.* 1973; Francisco *et al.* 1987; Forestieri *et al.* 1996). In the recent study by Siddiqui *et al.* (2007), a new triterpenoid (lippiacin) and a benzofuranone rengyolone (halleridone) was isolated for the first time from the methanolic extract of the *L. nodiflora* aerial parts.

The antibacterial activity may be due to several agents, such as the different solvent extracts or the presence of alkaloids, flavonoids, tannin, and oil as reported by Brantner (1996) and Irobi (1994). The antifungal activity of extracts in this research is in accordance with Tatsadjeu's studies (2009) on the leaf extract of *L. rugosa* against Aspergillus flavous. Antifungal activity was also studied by Viollon and Chaumont (1994). They used extracts of *L. multiflora* and *L. chevaliori* on flavous growth. They reported that terpenoides, particularly citral, geraniol and citronelol showed the most antifungal activity. Since there are some kinds of terpenoides in the *L. nodiflora* composition, we can say that there is antifungal activity in *L. nodiflora*. Linde (2010) showed high antifungal activity in *L. rehmannii* due to the presence of the oil contents β-caryophyllene and β-caryophylleneOxide, which were the major compounds that are in *L. nodiflora* as Sesquiterpenes (Pascual *et al.* 2001).

There are numerous studies about the antibacterial activity in species of *Lippia. L. origanoides* have high antibacterial activity due to the presence of mono terpenoides. The drop diffusion method showed highly significant inhibition zones for all microorganisms tested – gram positive bacteria (*S. aureus*) and fungus (*C. albicanse*) (Oliveria *et al.* 2007). In the research of Bassole (2003) on leaves of *L. multiflora* and *L. chevalievi*, gram negative bacteria were very sensitive. Khalil *et al.* (2007) used a thin layer chromatography (TLC) examination of the methanolic extracts of *L. nodiflora*, and reported that this plant containing a group of flavonoids. According to some researchers, flavonoides have antibacterial activity (Pascual *et al.* 2001).

Summarizing, leaves and flowers extracts of *L. nodiflora* showed antimicrobial activity to 4 gram negative, 4 gram positive bacteria and 2 fungi. Leaf and flower extracts showed similar activity. The observed activity could be due to the presence of flavonoides, terpenoides, sesquiterpenoids, phenolic acid, alkaloids and other components.

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