

Anti-inflammatory and antibacterial activities of *Lippia nodiflora* and its effect on blood clotting time

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Abstract

Aqueous extract of *Lippia nodiflora* possessed anti-inflammatory activity in Carrageenan induced mice paw edema in both doses 100 and 200 mg /kg bw ($P < 0.01$). However, the ethanolic extracts didn't induced anti-inflammatory effects in both doses. Aqueous extract of *Lippia nodiflora* exerted a concentration – dependent antibacterial activity against *E.coli* but not effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Ethanolic extract possessed antibacterial activity against gram positive (*Staphylococcus aureus*) and gram negative (*E. coli*) but not effective against *Pseudomonas aeruginosa*. Ethanolic extract of *Lippia nodiflora* significantly hasten blood clotting when used in a dose of 100 mg/kg ($P < 0.05$) and 200 mg /kg ($P < 0.01$). The effect of the ethanolic extract appeared dose dependent. However, aqueous extract didn't exerted significant effects on blood clotting time in both doses used in this study.

Keywords: anti-inflammatory, antibacterial, *Lippia nodiflora*, clotting time, experimental

التأثير المضاد للالتهاب والمضاد للبكتيريا لنبات الـ *Lippia nodiflora* وتأثيره على وقت تخثر الدم

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الخلاصة

كان للخلاصة المائية لنبات *Lippia nodiflora* تأثيرا مضادا للالتهاب في الاستسقاء المحدث بالكأراجينان في اخمص قدم الفئران عندما اعطيت بجرعة 100 و 200 ملغم / كغم من وزن الجسم ($P < 0.01$). على اي حال لم تحدث خلاصة الايثانول تأثيرا مضادا للالتهاب بكلتا الجرعتين . كما اظهرت الخلاصة المائية فعاليتها (معتمده على التركيز) ضد بكتريا الاشيريكية القولونية فيما لم يكن لها تأثير على بكتريا المكورات العنقودية الذهبية وبكتريا الزوائف الزنجارية . فيما احدثت الخلاصة الايثانولية تأثيرا مضادا لكل من بكتريا المكورات

العنقوديه والاشيريكيا القولونيه ولم يكن لها تأثيرا على بكتريا الزوائف الزنجاريه . ان الخلاصه الكحوليه للنبات عجلت في تخثر الدم عندما استخدمها بجرعة 100 ملغم / كغم ($P < 0.05$) و 200 ملغم / كغم من وزن الجسم ($P < 0.01$) . فيما لم تحدث الخلاصه المائيه تأثيرا معنويا على وقت تخثر الدم في كلتا الجرعتين المستخدمه في الدراسه .

Introduction

Lippia nodiflora (*Phyla nodiflora*) is a small perennial herb belonging to the family verbenaceae. It was distributed all over the world particularly in Africa, sub-continent and most of the tropical and subtropical regions, particularly in maritime areas close to rivers⁽¹⁾.

Lippia nodiflora contained triterpenoids, phenols (lippiflorin A and lippiflorin B, nepetin, batalilfolin, 6- hydroxyluteolin-7-Oapioside and luteolin-7-O-glucoside, flavones 6-hydroxyluteolin, Hispidulin 7-sulfate, hispidulin 7,4'-disulfate, jaceosidin 7,4'-disulfate, nepetin 3',4'-disulfate, nodifloretin 6,7-disulfate, 6- hydroxyluteolin 6, 7-disulfate, nodifloretin 7-sulfate, 6-hydroxyluteolin 6-sulfate, 6-hydroxyluteolin 7- sulfate, jaceosidin 7-sulfate, nepetin 7-sulfate, and hispidulin 4'-sulfate), steroids, nodifloretin, β -sitosterol glycoside, stigmasterol glycoside, nodifloridin A and nodifloridin B, lactose, maltose, glucose, fructose, and xylose. Halleridone and Hallerone as their acetyl derivatives were also from the leaves of *L. nodiflora*⁽²⁻⁷⁾.

L. nodiflora was reported to possess anti-inflammatory, analgesic, antipyretic⁽⁸⁾, antibacterial⁽⁹⁻¹²⁾, antifungal⁽¹³⁻¹⁴⁾, larvicidal⁽¹⁵⁾, antitumor⁽¹⁶⁾, antidiuretic⁽¹⁷⁾, antidiabetic, hypolipidemic⁽¹⁸⁾, and many other pharmacological effects. This study was designed to investigate the anti-inflammatory and antibacterial of *Lippia nodiflora* and its effect on clotting time.

Materials and Methods

Plant extraction:

The plant leaves were purchased from local market, diagnosed, dried, and powdered using pestle and mortar. Then 200 g of the ground leaves were divided into two equal portions and subjected

to exhaustive soxhlet extraction in ethanol or distilled water (500 ml each) for 72 h at 60 °C. Then the extracts were dried under vacuum⁽¹⁹⁾.

Experimental animals:

Sixteen Adult albino mice (weighing 25- 30 g) were used in this study. All the animals were housed in a cross ventilated room (temperature 22 $\pm$ 2°C, 12h light/12h dark cycle) and standard diet and water were given *ad libitum*.

Anti-inflammatory test:

Anti-inflammatory activity was assessed in mice on the basis of the inhibition of the Carrageenan induced hind paw oedema. Thirteen mice were divided into five experimental groups (6 animals each) and the basal thickness of the right hind paw was determined before the administration of any drug. Extracts (100, 200 mg/kg), and vehicle control were orally administered 1hr prior the Carrageenan administration. Acute inflammation was produced by the subplanter administration of 0.05 ml of 1% Carrageenan suspension in 0.9% NaCl in the right hind paw of the mice. The volume at the oedema was monitored by measuring the thickness at hind paw swelling 1hr after Carrageenan injection by using vernier caliper. The results are presented as the paw thickness variation in relation to the basal values⁽¹⁹⁻²⁰⁾.

Antibacterial test:

In vitro antibacterial effects was carried out on clinical isolates, Gram positive (*Staphylococcus aureus*) and Gram negative (*Echerichia coli* and *Pseudomonas aeruginosa*). The cultures of these bacteria were checked for purity by doing gram staining and biochemical test⁽¹⁹⁾, and they were grown in nutrient broth at 37°C and maintained in nutrient agar slants at 2-8°C. Nutrient agar medium

was used as bacterial culture medium in the antibacterial assays⁽²¹⁾.

Clotting time test:

Thirteen mice were divided in five groups (6 mice each). The first group was given the vehicle to serve as control. The rest four groups were given the aqueous and ethanolic extracts of *Lippia nodiflora*, each extract in two dose level (100 and 200 mg/kg bw) orally. one hour later, the time required for clotting of a drop of blood taken on slide by tail venipuncture was estimated according to the previous methods^(19, 22).

Results

Aqueous extract of *Lippia nodiflora* possessed anti-inflammatory activity in Carrageenan induced mice paw edema in both doses 100 and 200 mg /kg bw ($P < 0.01$). However, the ethanolic extracts

didn't induced anti-inflammatory effects in both doses (table 1).

Aqueous extract of *Lippia nodiflora* exerted a concentration – dependent antibacterial activity against *E.coli* but not against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Ethanolic extract possessed antibacterial activity against both gram positive (*Staphylococcus aureus*) and gram negative (*E. coli*) but not against *Pseudomonas aeruginosa* (table 2).

Ethanolic extract of *Lippia nodiflora* significantly hasten blood clotting when used in a dose of 100 mg/kg ($P < 0.05$) and 200 mg /kg ($P < 0.01$). The effect of the ethanolic extract appeared dose dependent. However, aqueous extract didn't exerted significant effects on blood clotting time in both doses used in this study (table 3).

Table 1: The anti-inflammatory effects of aqueous and ethanolic extracts of *Lippia nodiflora* in Carrageenan induced mice paw edema (mm).

Groups		Thickness of right paw before administration of Carrageenan (mm)	Thickness of right paw after administration of Carrageenan(mm)	The variations in the hickness of the paw between before and after administration of Carrageenan (mm)	Level of significancy in comparison with control group
Control		1.7±0.45	2.83±0.15	1.2±0.21	
Aqueous Extract	100mg/kg bw	2.12±0.22	2.90±0.33	0.75±0.23	$P < 0.01$
	200mg/kg bw	1.64±0.32	2.22±0.35	0.58±0.13	$P < 0.01$
Ethanolic Extract	100mg/kg bw	2.00±0.45	2.96±0.20	0.97±0.28	NS
	200mg/kg bw	2.06±0.41	3.03±0.25	0.97±0.20	NS

Table 2: The means of diameters of the zones of growth inhibition (mm) of 4 and 8 mg of aqueous and ethanolic extracts of *Lippia nodiflora*

Type of bacteria	Diameters of the zones of growth inhibition (mm)			
	Aqueous extract		Ethanolic extract	
	4 mg	8 mg	4 mg	8 mg
<i>Staphylococcus aureus</i>	-ve	-ve	4	8
<i>E. coli</i>	10	14	5	5
<i>Pseudomonas aeruginosa</i>	-ve	-ve	-ve	-ve

Table 3: Effects of aqueous and ethanolic extracts of *Lippia nodiflora* on bleeding time in mice

Groups		Bleeding time	Level of significancy in comparison with control
Control		87.0±31.0	
Aqueous extract	100mg/kg bw	104.0±13.5	NS
	200mg/kg bw	85.6±4.0	NS
Ethanolic extract	100mg/kg bw	66.0±5.9	P< 0.05
	200mg/kg bw	52.0±6.4	P< 0.01

Discussion

Plants are a main source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives. Medicinal plants are the Nature's gift to human beings to help them pursue a disease-free healthy life. Plants have been used as drugs by humans since thousands of years ago⁽¹⁾.

From our results, the aqueous extract of *Lippia nodiflora* exerted anti-inflammatory effects in Carrageenan induced mice paw edema. These results were in agreement with the results recorded by Ahmad *et al*⁽²³⁾.

Oedema formation in the paw was occurred as a result of synergism between various inflammatory mediators that increase vascular permeability and the mediators that increase blood flow⁽²⁴⁾. Although, inflammatory response can be induced

by several experimental models of paw oedema , but Carrageenan-induced paw oedema is widely used for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin were the first detectable mediators in the early phase of carrageenan-induced inflammation and prostaglandins were the mediators of the late phase of inflammation⁽²⁵⁻²⁶⁾. According to our results, it appeared that the aqueous extract of *Lippia nodiflora* the mediators of early phase of inflammation.

Antibacterial activities of the methanolic extracts from the leaves and flowers of *Lippia nodiflora* L. (Verbenaceae), were studied by Zare *et al* The extracts showed antimicrobial impact on bacteria such as *Bacillus subtilis*, *B. cereus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *K. oxytoca* and *Escherichia coli*. Our results showed

that increasing concentrations of extracts increased the antibacterial activities in all of the microorganisms. Bacteria were more sensitive than fungi, and gram positive bacteria were more sensitive than gram negative ones⁽²⁷⁾.

This study in part in agreement with other results which investigate the antibacterial and anti-inflammatory effects of *Lippia nodiflora*⁽⁸⁻¹²⁾, but our results showed that the alcoholic extract was more potent in Gram negative, these could be attributed to different types of extractive materials and different types of solvents. Zare *et al* extracted leaves and flowers by methanol while we extracted the leaves only by ethanol. The antibacterial activity may be due to alkaloids, flavonoids, tannin and oils of *Lippia nodiflora*, the same constituents when isolated from other plants showed antibacterial activity⁽²⁸⁻²⁹⁾.

The study of Ahmad *et al* recorded that the significant anti-inflammatory activity of the *Lippia nodiflora* extracts was observed in the first phase of carrageenin induced inflammation They suggested that this effect attributed to inhibition of early mediators, such as histamine and serotonin . Its effects on the second stage of inflammation was less⁽²³⁾, which give an indication that it has no strong effect on prostaglandins. Prostaglandins especially thromboxane A2 are the mediators which induced platelets aggregation and subsequent clotting. This could be explain the inhibitory effects of *Lippia nodiflora* on the clotting process and the prolongation of clotting time.

According to these results, It can be concluded that *Lippia nodiflora* exerted anti-inflammatory and antibacterial effects as well as, it enhanced clotting process and decreased blood clotting time.

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