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Evaluation of the Gastric Antiulcer, Antimicrobial and Antioxidant Activities of the Essential Oil from *Ocimum minimum* Linn.

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SUMMARY. Essential oil extracted from the leaves of *Ocimum minimum* Linn was characterized by chemical and physicochemical methods and its antiulcerogenic activity was determined. The chemical composition of the oil determined by using gas liquid chromatography associated to mass spectroscopy presented: 1,8-cineole, linalool, camphor, 2-terpineol, estragole, acetic acid, (-) β-elemene, α-bergamotene, α-humulene, β-cubebene, γ-cadinene and T-cadinol. The acute toxicity of the essential oil from *O. minimum* in mice by oral via was found at a dose of 1.5 mL/Kg. The antiulcerogenic activity presented by the essential oil in gastric ulcer induced by indometacin was detected by the reduction of the gastric lesion in 83.80% and induced by ethanol showed a reduction of 70.0%, both at the same dose (0.125 mL/Kg). Also, the oil presented an antioxidant activity when the sample was diluted 10 times (4.69 µmol/1h and 5.92 µmol/2h) which was quite similar to the control (6.29 µmol/1h and 6.16 µmol/2h). The antimicrobial activity of the essential oil was evaluated by the microorganism inhibition growth compared to ciprofloxacin as standard. The highest inhibition halos detected by using the oil was smaller (17 mm) than the highest one exhibited by using the antibiotic (35 mm). From the results it is possible to conclude that the essential oil from *O. minimum* has the significant antiulcerogenic activity concerning to the gastric lesion induced by indometacin and ethanol, as well as, significant antioxidant activity.

RESUMEN. "Evaluación de las actividades antiulcerógenas, antimicrobianas y antioxidantes del aceite esencial de *Ocimum mimum Linn."* El aceite esencial extraído de hojas de *Ocimum minimum* Linn fue caracterizado por métodos químicos y fisico-químicos y su actividad antiulcerogénica fue determinada. La composición química del aceite usando cromatografia de gas líquido asociada a espectofotometría de masas reveló la presencia de 1,8-cineol, linalol, alcanfor, 2-terpineol, estragol, ácido acético, (-) β-elemeno, α-bergamoteno, α-humuleno, β-cubebeno, γ-cadineno y T-cadinol. La toxicidad aguda del aceite esencial de *O. minimum* en ratones fue determinada por via oral en la dosis de 1,5 ml/kg. La actividad antiulcerogénica presentada por el aceite esencial sobre una úlcera gástrica inducida por indometacina fue detectada por la reducción de la lesión gástrica en un 83,80 %, e inducida por etanol mostró una reducción de 70,0%, ambos con la misma dosis (0,125 ml/kg). El aceite también presentó una actividad antioxidante cuando la muestra fue diluida diez veces (4.69 µmol/1h y 5.92 µmol/2h), lo cual fue muy similar con el control (6.29 µmol/1h y 6.16 µmol/2h). La actividad antimicrobiana del aceite esencial fue evaluada por la inhibición del crecimiento de microorganismos comparada con la ciprofloxacina como estándar. La mayor inhibición de halos detectada con el uso del aceite fue menor (17 mm) que la mayor exhibida por el uso del antibiótico (35 mm). Basándose en los resultados es posible concluir que el aceite esencial de *O. minimun* posee una significativa actividad antiulcerogénica en lesiones gástricas inducidas por indometacina y etanol, así como también una significativa actividad antioxidante.

INTRODUCTION

Ocimum minimum L. is a native species from India, where it is cultivated almost like holy plant. It is an herbaceous plant, aromatic, erect, very branched, with simple leaves, opposites, elliptic-oval, aerial parts in summit spiciforms and violet flowers, labiates [1] The stem is generally square and present hairs in the epidermis of the leaves and of the branches where the essence is found [2]. In popular medicine it is used as digestive stimulant, carminative, antipyretic, diuretic and anti-rheumatic. The leaves

KEY WORDS: Antiulcer, Antimicrobial, Antioxidant, Essential oil, *Ocimum minimum.*
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are the part of the plant used [1]. In Brazil two species are very used in the phytotherapy: *O. gratissimum* as mouth antiseptic and *O. basilicum* for stomach problems.

Among the contributions given by the medicinal plants from the *Ocimum* genera regarding to antiulcer action, the reduction of the incidence of gastric ulcer induced by aspirin was already observed with the ethanolic extract from *O. sanctum* [3]. The antiulcer action was also observed with banana fruit which acts inhibiting peptic ulcer [4] and Melia azedarach in ulcer induced by stress in mice [5]. Antimicrobial activity was the other effect observed by many medicinal plants which have too much contributed in complementary therapy as antimicrobials, such as: *Cariophylus aromaticus* [6], *Aloe vera, Capraria biflora* L.; *Eucalyptus citriodora; Zingiber officinale* and *Psidium guayava* [1]. The aim of this work was to investigate antiulcer, antioxidant and antimicrobial activities, as well as acute toxicity of the essential oil obtained from *O. minimum* Linn.

MATERIALS AND METHODS

Plant material

Leaves of *O. minimum* were obtained from the plant garden of the "Universidade Estadual Vale do Acaraú", Sobral, Ceará, Brazil. A voucher specimen was placed in the "Prisco Viana" Herbarium at the "Universidade Federal do Ceará", Fortaleza, Ceará, Brazil, with the number 30852.

Animals

Swiss mice of both sex were obtained from the animal room at the "Laboratório de Imunopatologia Keizo Asami (LIKA)/Universidade Federal de Pernambuco", Brazil and all of them were mantained at the same environmental conditions (temperature of 25 °C, clear/dark) provided with water and food *ad libitum*.

Male Wistar rats (200-300 g) were supplied by the "Departamento de Fisiologia e Farmacologia/Universidade Federal de Pernambuco", Brazil and mantained at the same conditions as before.

Microorganisms used were: *Staphylococcus aureus, Escherichia coli, Salmonella enteritidis, Pseudomonas aeruginosa, Bacillus cereus* and *Candida albicans*. They were supplied from the microorganisms collection of the "Departamento de Antibióticos/Universidade Federal de Pernambuco", Brazil.

Extraction of the essential oil from O. minimum leaves

The oil was extracted by using vapor dragging and water distillation. The material was placed in a container and a water vapor current pass through it under pressure. The volatile products present in the leaves were dragged by the water vapor and the mixture was taken to a condenser where the vapors returned to liquid state and were collected in a separator flask. The essential oil is a mixture of insoluble organic substances in water, which was isolated by using hexane resulting in a two-phases system [2]. Then the material hydrolyzed was dried in a rotatory evaporator under vacuum at 40 °C.

Chemical Characterization of the Essential Oil

The chemical constituents of essential oil were identified by comparing to the mass spectra obtained with standards. The conditions were: DB-5-dimethylpolysiloxone column (30 mm x 0.25mm); N_2 gas; temperature 35-280 °C, injection volume 1 µL The program used was developed by A.A. Craveiro and J.W. Alencar [2].

Acute toxicity of the Essential Oil

Groups of six mice, three male (32.00 g) and three female (26.00 g) were used to determine LD_{50} to the which crescent doses of 0.25; 0.50; 1.5 and 5.0 mL/Kg were administered by oral via. The percentage of death was observed during 72 h and the DL_{50}% was calculated using probit method according to Miller & Tainter [7] model.

Indomethacin-induced gastric ulcers

The antiulcer activity was evaluated as described by Hayden [8]. Four groups of six rats (150 g) were maintained in fasted for 24 h water *ad libitum*. Sixty min before of the indomethacin injection (20 mg/Kg; s.c.), 18 animals were treated by gavage with oil in the following doses: 0.125; 0.250 and 0.500 mL/Kg. Oil doses were repeated three h after the anti-inflammatory to be administered. After 6 h from the first dose, the animals were sacrificed under anesthesia with ether. The stomachs were removed and opened, washed with water and placed in Petri dishes for the gastric lesions inspection. The ulcer degree for the each stomach was established according to the follow classificatory scale:

Loss of the mucosa pleat	1 point
Loss of the mucosa color	1 point
Edema	1 point
Hemorrhage	1 point
Number of spots (up 10)	2 points
Number of spots (more than 10)	3 points

Ulceration intensity was determined according to the following scale (n = number of ulcers found)

Ulcer or erosion up to 1 mm	n x 2 points
Ulcer or erosion more than 1 mm	n x 3 points
Ulcer perforated	n x 4 points

Ethanol-induced gastric ulcers

Five groups of six rats (310g) male in fasted for 24 h were treated with 0.9% salt (5 mL/Kg), plant oil (0.025; 0.040; and 0.060 mL/Kg, p.o.) and dexchlorpheniramine (3mg/Kg, p.o.). After 45 min 50% ethanol (5 ml/Kg, p.o.) was administered to induce the gastric ulcer [9]. One hour after the animals were sacrificed by anesthesia with ether. The stomachs were removed and inspected the gastric lesions as procedure described for the previous model.

Antioxidant activity of the essential oil

Macrophages culture

Macrophages (2 million), 2 mL RPMI 1640 medium completed with 3% fetal bovine serum in both control and samples and essential oil prepared in methanol (100 µL) were placed In cell culture plates. The mixture was incubated in stove at 37 °C with [CO_2] approximately 5%. After 1 h the medium was discarded, cells were washed with 1 mL of RPMI medium, 2 mL of RPMI medium without 3% bovine fetal serum was added in each role and after 1 h the macrophages were available to be used in the next experiment.

Production and release of superoxide radical (O^-_2):

In the culture plates 29 µL of the superoxide dismutase enzyme which was used as positive control and 29 µL of distilled water as a negative control were added. The mixture was incubated at 37 °C for 10 min and 143 µL of cytochrome c and 2.145 µL of PMA (phorbol miristate acetate in Hanks) were added. Aliquots of 600 µL were immediately collected (samples and blank) at 1h and 2h, the material collected was centrifuged at 10,000 x g for 5 min and absorbances of the supernatant were measured at 550 nm.

Antimicrobial activity of the essential oil

The antimicrobial activity of the essential oil from *O. minimum* L. was evaluated according to method described by Bauer [10]. This method is based in the microorganism growth inhibition which is detected by the disc diffusion technique.

Paper discs preparation

Filter paper discs of 30 mg ± 4 mg/cm^2 and 6 mm of diameter were soaked with 20 µL of essential oil from *O. minimum*. Ciprofloxacine (20 µL) was used as standard.

Culture media preparation

Mueller-Hinton medium was used for bacteria culture maintenance and Sabouraud medium for yeast. These cultures were diluted with 0.45% of salt following the Mac Farland scale (10^8 UFC/mL).

Culture plates preparation

Mueller-Hinton solid medium (18 mL) was placed in Petri plates (90 mm of diameter) until complete solidification.

Inoculum preparation

Microorganisms suspensions were spread on the medium surface. Paper discs impregnated with essential oil were placed on the medium surface.

Measurement of inhibition halos

Inhibition halos were measured after 18 h of incubation using a ruler and the halos diameter of the oil were compared with those of ciprofloxacin.

Statistic analysis

The results obtained in all analysis were expressed in media ± SD (standard deviation). The levels of statistic significance ($p<0.01$ and $p<0.05$) were calculated based in Student T Test.

RESULTS

The essential oil extracted from the leaves of the *O. minimum* plant by hydrodistillation and vapor dragging and solubilized in hexane presented yellow color, aromatic smell and density of 0.9256 g/mL and a yield of 2.3%

Figure 1 shows the mass spectrum of the essential oil chemical constituents. It can be seen that the oil presented in its chemical composition derivates according to Brady [11] of terpene formed by mevalonic acid acetate via, such as: 1.8-cineole, linalool, camphor, 2-terpineol, estragole, acetic acid, (-)β-elemene, α-bergamotene, α-humulene, β-cubebene, γ-cadinene, T-cadinol. These constituents present in its struture the following chemical groups: hidrocarbonetos, alcohols, ketones, ethers and oxydes.

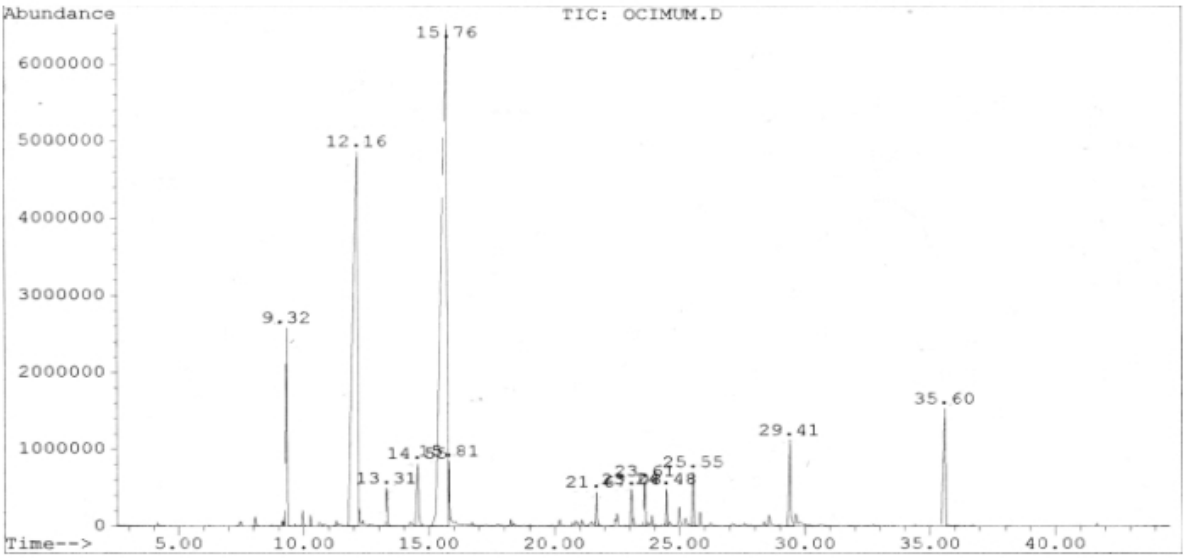

Figure 1. Elution profile of the essential oil chemical constituents from *O. minimum* performed by using gas liquid chromatography associated to mass spectroscopy.

The LD_{50} of the essential oil from *O. minimum* found in mice by oral via was 1.5 mL/Kg. At dose of 1.5 mL/Kg and 5.0 mL/Kg, p.o., the essential oil was CNS depressant in animals which were observed changes in spontaneous motor activity, reactivity to sound and touch.

The effect of essential oil from *O. minimum* on gastric lesions induced by indomethacin can be observed in Table 1. The reduction of the gastric lesions was in media 83.80% of inhibition ($p<0.01$) which was observed by using the oil in the doses of 0.125, 0.250 and 0.500 mL/Kg.

The effect of the essential oil from *O. minimum* (0.125mL/Kg) in the gastric lesion induced by ethanol presented very significative values as can be seen in Table 1.

The scores media showed that the group treated with 0.125 mL/Kg of oil reduced the lesions induced by ethanol in 70%.

The essential oil as raw extract did not present antioxidant activity, however this effect was observed when this material was diluted 10 times (Table 2).

It can be observed from the inhibition halos diameter in Table 3 that the microorganisms growth are less inhibited by the oil than by the ciprofloxacin used as antibacterial standard.

DISCUSSION

The mass spectra of *O. minimum* essential oil was similar to that found by Craveiro [1] for the *O. gratissimum* essential oil. These results were also comparable with those found by Amparo [12] for the *O. basilicum* essential oil, however it presented different flavonoids profile.

Concerning to LD_{50} the toxicity data showed that the essential oil was quite non toxic, having a LD_{50} value of 1.5 mL/kg. At doses 1.5 mL/Kg and 5.0 mL/Kg, it was CNS depressant with respect to reactivities to sound and touch and spontaneous activity. These doses are respectively 12 and 20 times higher than that one

Group	Dose (mL/kg; p.o.)	Ethanol (ulcer score)	Indomethacin (ulcer score)
Saline 0.9%	5.000-	27.80±5.80	39.5±20.74
EOOM	0.041	23.33±5.60 (16.0%)	–
EOOM	0.062	22.83±5.41 (18.0%)	–
EOOM	0.125	8.33±5.57 (70.0%)*	6.4±3.84* (83.8%)
EOOM	0.250	–	9.2±7.15* (76.7%)
EOOM	0.500	–	6.4±4.10* (83.50%)

Table 1. Effect of *O. minimum* essential oil on indomethacin induced ulcers in rats. EOOM = Essential oil from *O. minimum*. *$p<0.05$; ( ) Percentage of inhibition.

System	Absorbance		Superoxide Concentration (µmol)	
	1h	2h	1h	2h
Control	0.051	0.050	6.29	6.16
Pure sample	0.107	0.122	13.19	15.04
Diluted sample (1/10)	0.038	0.048	4.69	5.92

Table 2. Evaluation of antioxidant activity of the essential oi from *O. minimum*. The formule below is used to calculate concentration of superoxide radical: K = 205,9 x Absorb. X 600. K is express in nmol or (mol of superoxide radical.

Microorganisms	Inhibition halo found with essential oil (mm)	Inhibition halo found with ciprofloxacin (mm)
Staphylococcus aureus IC-403	10	17
Staphylococcus aureus IC-159	10	31
Escherichia coli DAUFPE-24	10	35
Escherichia coli IC-02	10	30
Salmonella enteretidis DAUFPE-415	9	30
Pseudomonas aeruginosa IC-472	-	-
Pseudomonas aeruginosa IC-515	-	-
Bacillus cereus DAUFPE-11	24	35
Candida albicans DAUFPE-1007	17	NT
Candida albicans DAUFPE-IC-07	15	NT

Table 3.

which presented gastric protection (0.125 mL/Kg) in the experimental models used. These effects probably can be attributed to the highest concentrations of estragole (50.42%), linalool (30.57%) and 1,8-cineol (4.9%), which belong to chemical group ether and alcohol [13].

Indomethacin is a good prostaglandin syntase inhibitor and this action can be associated to its harmful effect in the gastric mucosa. The reaction must be attributed to the inhibition of the prostaglandin biosynthesis PGE_2 and PGI_2 (ciclooxygenase inhibition via arachydonic acid metabolism), with consequent increase in the production of leukotrienes and other products by the lypoxygenase pathway [14].

The gastric lesion observed in the group of rats treated with essential oil from *O. minimum* was of low intensity compared with control groups even being independent dose, because in the doses of 0.125 mL/Kg and 0.500 mL/Kg around 83.00% of inhibition was observed. However this result showed statistically significance (Table 1) and it suggests that the essential oil from *O. minimum* showed like a protector agent against this type of lesion. However, at this moment it is not possible to identify which mechanism involved in the pathogenesis of the gastric lesions induced by indomethacin would be influenced by the presence of the oil.

The mechanisms involved to induce gastric lesions by ethanol are complex and numerous, with venoconstriction, artery dilation, autacoids liberation [15]. According to Llesuy *et al.* [16], the ethanol increases superoxide anion and hidroxyl radical production and lipid peroxidation in the gastric mucosa. The antioxidant effect presented by the *O. minimum* essential oil can be probably one of the mechanism involved in the mucosa gastric protection.

Several studies have shown antioxidant activity of flavonoids and another natural compounds. The antioxidant activity of flavonoids is efficient in trapping superoxide anion ($O_2^{\bullet -}$), hidroxyl ($OH^{\bullet}$), peroxyl ($ROOH^{\bullet}$) and alcohoxyl ($RO^{\bullet}$) radicals [16].

The antioxidant effect non observed with the crude extract can be attributed to the high concentration of the substance related to the number of cells, however this effect was visible enough after sample dilution. The preparation of the crude extract with alcohol probably contributed to the absence of activity due to its aggressive effect to the cells meanwhile after dilution of the extract with culture medium the antioxidant activity could be evidenced.

According to the founds with this work it is possible to conclude that the essential oil from *O. minimum* has a significant protector effect against gastric lesion induced by indomethacin

and ethanol. However further studies are need to elucidate the action mechanism involved in the gastric protection and the antioxidant activity.

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