Antioxidant and wound healing potential of saponins extracted from the leaves of Algerian *Urtica dioica* L.

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Abstract: The Nettle is a herbaceous and vivace plant of Asian origin. It is integrated in several areas especially alimentary, agricultural, industrial and medicinal. The aim of this work is to demonstrate through pharmacological tests a possible antioxidant and wound healing effect of crude saponins of the leaves of *Urtica dioica* L. The extraction method is based on the degree of solubility of saponins in organic solvents. The antioxidant activity of the leaves extracts was evaluated by the diphenyl-picryl-hydrazyl test (DPPH). The wound healing effect is *interpreted* on the basis of the healing time and the evaluation of the surface of wounds. It appears from this study that the Nettle is rich in saponins, either 4.08% to 30 g of plant powder. The results also showed significant antioxidant effect similar to that of ascorbic acid ($p > 0.05$) with an IC 50 of 0.159mg/ml. As regards the healing power, treatment of rats with the product based on crude saponins is achieved after 15 days, either 100% of wound reduction. This value is much higher than that obtained by the reference product (Madécassol®) on the same duration of treatment with 93.73% of wound reduction. The achievement of pharmacological tests has thus shown that crude saponins extracted from the leaves of *Urtica dioica* L. can be integrated into the pharmaceutical field or even in cosmetic.

Keywords: *Urtica dioica* L., saponins, antioxydant activity, MADÉCASSOL®, cicatrization.

INTRODUCTION

The plants represent a new source of active compounds. Indeed, secondary metabolites are and remain the subject of many researches in vivo and in vitro, especially the research of undiscovered natural compounds such as phenols, saponosids and essential oils (Bahorun, 1997), especially the search for new natural constituents such as phenolic compounds, saponins and essential oils (Bahorun, 1997). Among these plant species, nettle is widely used in traditional medicine. Around the world, many researchers are carried out on the Great nettle (*Urtica dioica* L.). It was used in Iran as a remedy for diabetes mellitus (Farzami *et al*., 2003); (Petlevski *et al*., 2003) allergic rhinitis and rheumatoid arthritis (Mittman, 1990; Riehemann *et al*., 1999) and in Morocco against cardiovascular disease (Legssyer *et al*., 2002). It has been reported that *Urtica dioica* has also been used in the treatment of pain (Akbay *et al*., 2003), prostate cancer (El Haouari, 2006) and in the case of a deficiency of neutrophils function (Basaran *et al.*, 1997). However, little researchers on the biological effects of Nettle are carried out in Algeria. The infrequent works are those of Megnounif (2011) on the nutritional value and antioxidant activity, and those of Bouali (2014) carried on the phytochemical characterization. This lack of information guides our interest for the characterization and measurement of biological and pharmacological properties of crude saponins extracted from the leaves of this medicinal plant, especially their antioxidant and wound healing effect. Indeed, many biological activities are associated with saponins, such as analgesic activity, anti-inflammatory, anticancer, hemolytic, antimicrobial, antiviral, insecticidal, diuretic and sedative (Lacaille; Wagner 1996).

MATERIALS AND METHODS

Preparation of plant material

Urtica dioica L. was collected in March 2014 in the Boumerdes region (Algeria) in the Forest of Bouarbi and recognized by Dr. Abdekrim, Taxonomist, Botany Department of the National Superior School of Agronomy (ENSA), Algeria. The leaves of the plant were then dried, crushed and stored in glass vials in the absence of light and moisture for subsequent analysis.

Test for the presence of saponins

The revelation of the presence of saponins in the decoction of 1% is accomplished according to the method described by Wagner *et al.* (2001), Bekro *et al.* (2007). For this, a series of 10 assay tubes containing increasing quantities of decoction, are adjusted to 10ml with distilled water. Previously prepared test tubes are stirred during 15 seconds with two stirs per second, and then allowed to stand for 15minutes. The height of 1cm of the foam

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indicates the richness of the plant on these secondary metabolites.

Preparation of the extracts

The extract is prepared according to the method described by Bruneton *et al.* (1999). Thus, seventy grams (60g) of plant powder of the leaves are macerated in 218ml of methanol for 72 hours with stirring at room temperature (25°C). The filtrate evaporated at 45°C using a rotary evaporator (Buchi 461) is mixed with 240 ml of distilled water and n-butanol (v/v). The dry extract obtained after evaporation of the organic phase is dissolved in 36ml of methanol and 180 ml of ethyl acetate. After 72 hours of maceration at room temperature, the filtrate is dried in rota vapor at 45°C. The obtained extract, recovered by 10 ml of methanol, constitute crude saponins.

Antioxidant activity

The evaluation of the antioxidant effect is realized by the scavenging of the free radical DPPH test, adopting the method described by Sanchez *et al.* (1998). This study is based on trapping the stable free radical DPPH by a scavenging molecule, causing its discoloration (Molyneux 2004). The method is quick and convenient to be implemented. It is carried out at ambient temperature, to eliminate any risk of thermal degradation of the molecules tested. For this, a volume of 25µl of each methanolic extracts at various concentrations (0.0125 to 1mg/ml) is added to 1.95ml of the methanol solution of DPPH (0,0024g/l). In parallel, a negative control is prepared by mixing 25µl of methanol with 1.95 ml of the methanol solution of DPPH. The reading of the absorbance is made against a blank prepared for each concentration at 517nm after 30min incubation in the dark and at room temperature. The positive control is represented by a standard solution of an antioxidant (ascorbic acid), whose absorbance is measured under the same conditions as the samples. The test is repeated three times for each concentration.

The results are expressed as percent inhibition (I %) according to the following formula:

$$I\% = \frac{(\text{A reference} - \text{A test})}{\text{A reference}} \times 100$$

Where:

A reference: the absorbance of the control
A test: the absorbance of the extract

The IC50 values are determined graphically by linear regression.

Study of the healing activity

In our study, we followed the method of Poisot and Dumez, (1978). The aim of this study consists in the application of the test product on provoked wounds. It consists on pomade prepared by mixing two grams (2g) of leaf extract (crude saponins) with ten grams (10g) of Vaseline which is equal to 20% of concentration. The test is performed in six male Wistar rats in good health, weighing between 200 and 230grams, acclimated for a week prior to the experiments in the animal house of the Department of Pharmaco-toxicology of pharmaceutical group SAIDAL (Algiers). The rats, divided into two batches, are anesthetized with an intramuscular injection of ketamine hydrochloride at a dose of 15mg/kg. The hairs from the back of the animal were shaved with an electric shaver. Then, two wounds of two centimeters are provoked on either side of the lumbar column of each rat. In order to minimize the risk of error, only one wound is treated, the other is left as control. Thus, each animal served as its own control. The two groups of rats were treated respectively with Madécassol® (reference product) and the pomade. The treatments are applied once a day during 15 days. It should be mentioned that the wounds are not protected by a bandage. The footprints wound surfaces are taken first on transparent paper, then every two days. The interpretation of the results is based on the comparison of the evolution of the wounds surfaces and was realized using AUTOCAD® software. The percentage of the reduction of the wound surface is calculated according the following formula:

$$\%\ \text{of reduction(product}\ \mu\text{CE)} = \frac{d0 - dn}{d0} \times 100$$

Where:
d0: wound surface at d0
dn: wound area on day dn

STATISTICAL ANALYSIS

The results are statistically analyzed using Student's test. All values are expressed as mean ± standard error at the mean. The significance level was set at $p < 0.05$.

RESULTS

The yield of extraction

The saponin extract obtained presents a liquid appearance and a dark green color. The yield of crude saponins obtained from 30g of plant powder is 4.08%, which shows the richness of Nettle in these metabolites

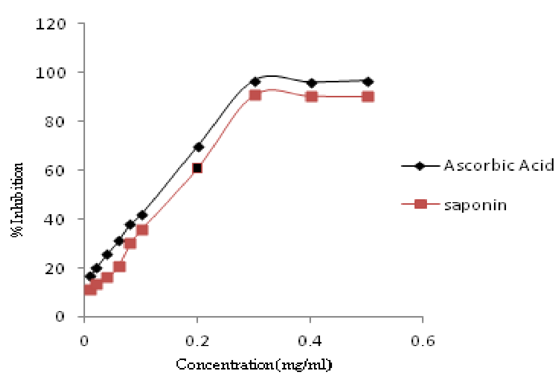

Fig. 1: Percent inhibition (I%) of different concentration of the extract saponin and ascorbic acid.

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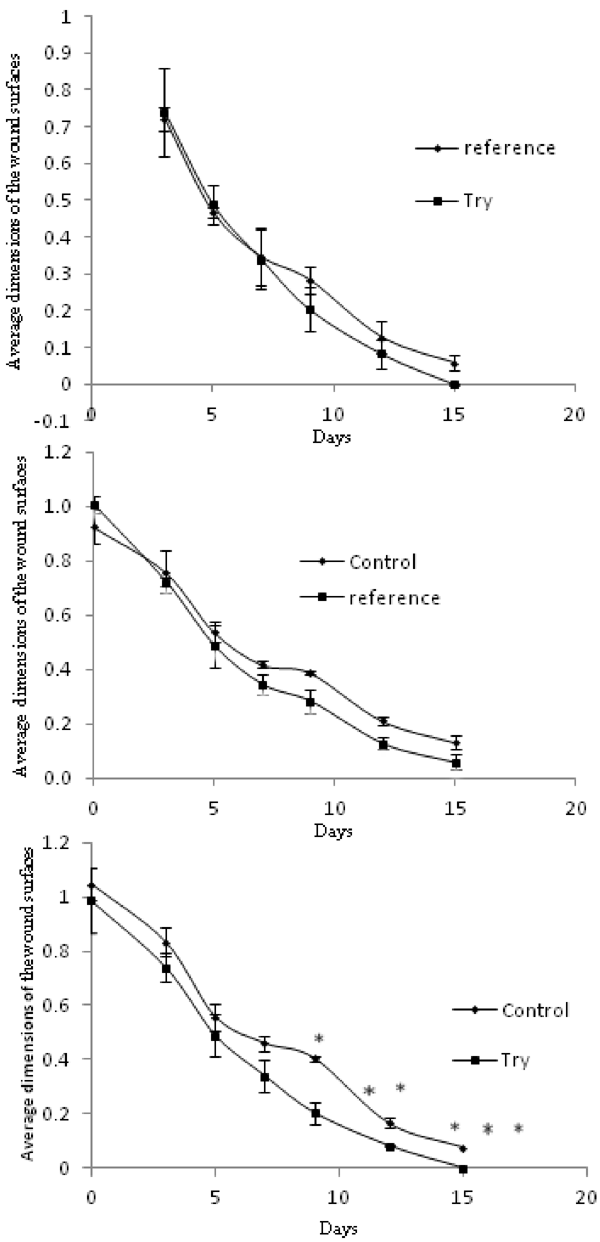

Results are expressed as mean ± standard deviation. Student's test:* P <0.05, ** p <0.01; *** P <0.001; T: treated group compared to controls. (R = reference, C= Control, T= tries).

Fig. 2: Representative curves of the lower average surface (cm^2) of the wound in function of time.

Determination of hydrogen donation ability (DPPH test)

The percentage inhibition of the free radical DPPH increases with increase in the concentration either to ascorbic acid or to the saponin extract. The results show that the percentage inhibition of the free radical of saponin extracts of *Urtica dioica* L. is very similar to that of the used standard (ascorbic acid) for all concentrations tested (fig. 1). The difference between the percentages of inhibition obtained to extract and ascorbic acid is not significant (p>0.05). The higher value of percentage inhibition for the saponin extract (90.75%) is noted for the concentration of 0.6mg/ml, against 96.75% for ascorbic acid at the same concentration.

The calculated IC 50 value of the saponin extract compared with that of ascorbic acid shows a very significant inhibitory power.

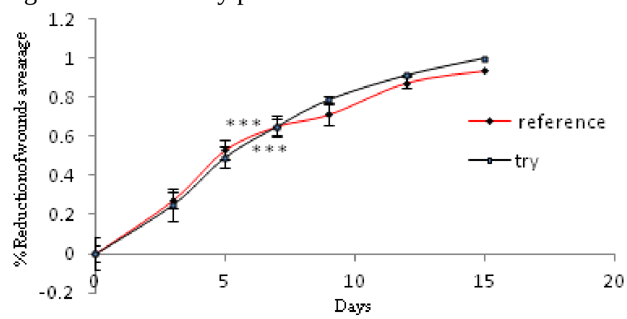

Fig. 3: Curve representing the percentage reduction in the average areas of the wounds versus time.

Healing activity

Evaluation of average dimensions of the wound surfaces

During the experimental period, no mortality was observed in animals. All rats were in good health, and were available for evaluation. The morphological parameters of wound surfaces were used to evaluate the healing effect of the saponin extract. fig.2 contain the values corresponding to the evolution of average dimensions of the wound surfaces.

Through the data obtained, we note a reduction in the average surface wounds in two lots but unevenly. The results of our study show that no significant change (p> 0.05) from the surface of wounds was observed during the first three days of the incision. Between the 5th and 7th day, the reduction of the average area of the wounds is the same for the different treatments. (p>0.05). From day 7, we observed an important and significant (p>0.05) reduction of the average area of wounds treated using the reference product comparing to those treated with other treatments. However, a significant reduction of the average surfaces of the treated wounds with the reference product compared with other treatments is observed from the 7th day (p<0.05). The healing activity of the saponin extract is more visible from the 9th day (P 0.05). Indeed, the recorded value is slightly higher than that of wounds treated with the reference product, respectively 0.203± 0.040 cm^2 and 0.284±0.042cm^2. Finally, in the twelfth and the fifteenth day, the average wound surfaces treated by saponin extract represent approximately half of that of the wounds treated with Madécassol®. The values are: 0.082 cm^2 against 0.128 cm^2 on the 12th day, and 0 cm^2 against 0.059 cm^2 on the 15th Day.

Percentage reductions of wound area

The evolution of the healing process of the observed wounds, compared with the control group in the 3rd, 5th,

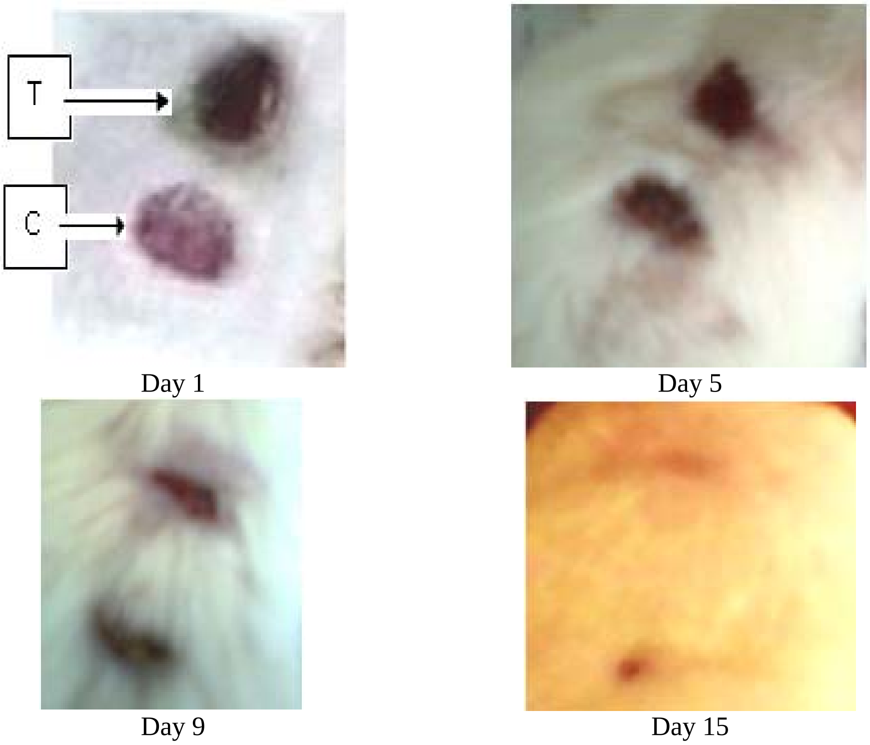

Fig. 4: Wounds untreated and treated with the pomade

7th and 15th day of treatment (fig. 3), clearly indicates that the reduced wounds was significantly potentiated in groups treated with saponin extract ($p < 0.05$). This healing process was less for wounds of rats treated with Madécassol®. In addition, it is important to note that during daily visual observations, we found the presence of signs of infection around the wound in the control animals. The wounds of rats treated with the saponin extract and those treated with Madécassol® were not infected. Finally, and despite the difference in the healing process of the wounds, the evolution of reduction and the time of closure wounds become statistically similar in the treatment of groups with Madécassol® or in the batch tries (fig. 3).

The obtained results show that the percentages reduction of wound surfaces for µCE2 test group are higher (100%) compared to the percentage reduction in wound area noted for the group µCE1 (93.73%) . It appears that crude saponins are endowed with healing power as important as Madécassol® pomade.

Evolution of the healing process

The healing process is been through a progressive disappearance of the inflammation phase and a contraction phase.

Wounds untreated and treated with the pomade

The wounds treated with the pomade prepared from saponin leaf extract of Nettle have healed after 15 days. The fig. 4 shows the images of the wounds treated with the pomade at D1 (creation of the wound), D9 (when the wound achieved 78% of shrinkage) and D15 (hair growth). In 15 days of treatment, the pomade has led to a total skin wound healing, tissue remodeling and hair regrowth. Untreated wound has healed after 18 days.

Wounds treated with the reference product

fig. 5 shows the images of the wounds treated with Madécassol® at D 9 (when the wound has attained 65% of shinkage) and at D15 (hair growth).

DISCUSSION

The presence of the saponins in the aerial part of the Stinging Nettle is indicated by Bazzaz, (1997); Kavtaradze *et al.* (2011); Manjir *et al*. (2012), collected respectively in Iran, Georgia and India. However, Safanah *et al.* (2012) and Ghaima *et al.* (2013) mentioned the absence of these metabolites in the aqueous extract of the aerial part of Nettle collected in Iraq. This difference is only the reflection of the impact of biotic and abiotic factors on the synthesis of these metabolites. Indeed, Ramakrishna and Gokare (2011), affirm that the synthesis of secondary metabolites in plants is influenced by biotic factors and abiotic factors .It has been shown that antioxidant molecules such as ascorbic acid, tocopherol, flavonoids, tannins and saponins reduce and discolor DPPH by reason of their ability to give hydrogen (De Pooter and Schamp, 1986). Due to lack of saponins

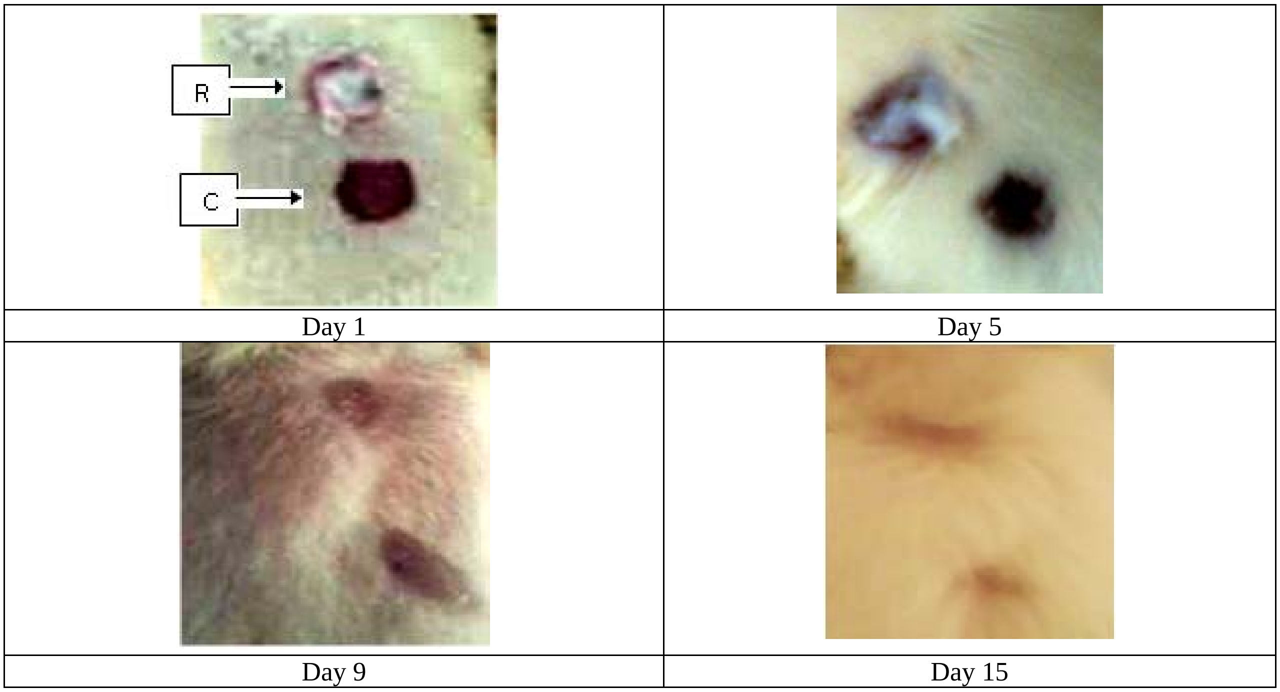

Fig. 5: Wounds untreated and treated with the reference

researchers on the Stinging Nettle, we have not been able to discuss these results.

In order to stimulate the process of wound healing, several drugs have their origins from plants. Habbu *et al.* (2007) have invested in a study providing a detailed review of the literature on natural healing, the different phyto constituents, the formulations of several plants and the various nutraceuticals responsible for wound healing activity. fig. 5 describing the evolution of the healing process showed that both products Madécassol ® and saponin extract of *Urtica dioica* significantly stimulate wound contraction (p <0.05) and allow of shortening the period of epithelialization. However, the second seems to be more effective than the first. Madécassol® is one of the commonly used products for the treatment of wounds. shetty *et al.* (2008) reported that the main ingredients of the titrated extract of *Centella asiatica* (Asiaticoside, Asiatic acid and madecassic) are effective in systemic sclerosis, formation of abnormal scars and also significantly shorten the time healing. Their most beneficial effect appears to be the stimulation of the maturation of scars due to the production of type I collagen and the reduction of the inflammatory reaction and the production of myofibroblasts. However, no studies have been published regarding the saponin extract of *Urtica dioica* L. The phenolic compounds have been identified as having antibacterial and antioxidant properties (Siger *et al.,* 2008).

It has been shown that plants which have healing and vulnerary properties have often a high level of plant sterols (Dweck, 2007). Various antioxidants are able to reduce free radicals, preventing the impairment at the cellular level. They inhibit inflammation, which leads to the depletion of collagen, and they provide protection against photonic damage and skin cancer. Finally, maybe the different properties of the components previously cited of this saponin extract, could explain its healing effect. The results obtained at the end of this study (illustrated in fig. 2) show a potentiation of the healing process from the seventh day of the operation in tries and reference groups compared to the control group, whose products effects are statistically indifferent. This inducing effect of wound healing is significantly better in animals treated with the saponin extract of Nettle with a less degree for the rats treated with Madécassol®, a reference healing agent. The beneficial effect of potentiating and saponin extract is clearly observed in the period between the 7th and 15th day of the operation, compared to other lots whose the healing process is clearly slowed down. The visual and daily observation of wounds excision showed the presence of signs of infection in the wounds excision of most rats except those treated with saponin extract. This observation can be interpreted by the presence of an antimicrobial power of saponin extract of Nettle. This explains the slight advantage of the observed effect of saponin extract of Nettle against the effect observed with the Madécassol® during the period of 7-15 days of healing.

The healing effect of the pomade prepared with crude saponin extract is more effective than the reference product (Madécassol®). It is important to note that the reference product Madécassol® contains very active constituents such as asiacoside and centelloside

(Brinkhaus, Lindner *et al.* 2000). According to this author, Madécassol® stimulates and regulates the production of collagen, fibrous matrix essential to wound healing.

CONCLUSION

The present study has demonstrated the presence of crude saponins endowed with antioxidant and wounds healing properties. The extracts of saponins seem to present a real and potential interest for their antioxidant activities that have been determined by the test (DPPH). The study of this plant, led to the conclusion that saponin leaf extract showed efficacy in caused wounds healing. In this work, the pharmacological properties of the leaves of nettle, justify their use in traditional herbal medicine and could indicate the possibility of therapeutic use as an antioxidant and healing. More research is needed to standardize the chemical composition of the saponin extract and understand the physiological mechanism behind his healing activity. Other toxicological tests seem more than necessary and other therapeutic virtues are to uncover in the hope of finding the saponin extract obtained from the leaves of *Urtica dioica* L, a place in modern pharmacology.

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