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Phytochemical screening and hemolytic activity of some leaves extracts of *Lantana camara* L.

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Abstract: Medicinal plants have several therapeutic properties; they have been used for a long time to treat different diseases. *Lantana camara* L. has been widely used by man for healing these diseases. In this study, four leaves extracts of *L. camara* were subjected to preliminary phytochemical screening to determine the presence and/or the absence of phytochemical constituents; In addition, they were tested for hemolytic activity on human erythrocytes. This activity is performed using the UV-Vis spectrophotometer method at 520 nm and at five different concentrations (125 µg/ml, 250 µg/ml, 500 µg/ml, 750 µg/ml, and 1000 µg/ml). The phytochemical screening showed the presence of various phytochemical groups such as phenolic compounds, saponins, sterols, tannins, flavonoids, reducing compounds and the absence of alkaloids in the four extracts. These same extracts showed average hemolytic activity sequentially: chloroformic extract, petroleum ether extract, aqueous extract and then methanolic extract. This activity is dependent on the concentration of the extract.

Keywords: *Lantana camara*, phytochemical screening, hemolytic activity, medicinal plants, organic extracts

Introduction

For a long time, humans have used plants for therapeutic purposes to treat various diseases such as fever, cold, pain, headache and others [1]. This use is reported in the oldest literature [2]. All the different organs of the plant such as leaves, bark, roots and flowers are used in the treatment of common diseases such

as bronchitis and tracheobronchite [3]; in fact, the plant is a very rich source of alkaloids, glucosides, saponins, essential oils or resin used for this purpose.

Lantana camara L. is an ornamental plant long used in the treatment of various diseases such as colds, varicella, ocular lesions, asthma, bronchitis and high blood pressure [2, 4, 5] it is mentioned as one of the most important medicinal plants in the world [4, 7, 8]. This plant belongs to the family of Verbenaceae native to tropical and subtropical regions. Currently, it occurs in various countries where it is often cultivated as an ornamental plant [9].

Studies on the phytochemical composition of this plant have shown its richness in secondary metabolites such as alkaloids, saponins, flavonoids, sterols, tannins and other compounds; in addition, studies on biological activities have shown that *L. camara* has several effects such as antibacterial effect [10], antifungal effect [11], anti-cancer effect [12], anti-inflammatory effect [13], wound healing effect [14], antioxidant effect [15] and a hyper-glycemic effect [16]; what makes it an important source of new compounds for the pharmaceutical industry and a future candidate for the discovery of new drugs [1]. In this context, the objective of this work is to evaluate the phytochemical composition of four leave's extracts of *L. camara* and to evaluate their hemolytic activity against human erythrocytes.

The choice of this plant was made based on the fact that it is one of the medicinal plants richest medicinal plants in phytochemicals compounds, and according to bibliographic research and our best knowledge, this is the first study of the hemolytic activity of this plant in Algeria.

Materials and methods

Collection of plant material

The plant material consists of the leaves of *L. camara* prospected in the region of Ferdjiwa, Mila, Algeria in November 2018. These leaves were washed with plain water and then dried in shade at room temperature. After drying, the leaves are reduced to a fine powder using an electric grinder.

Preparation of plant extracts

1- Extraction by water maceration

50 g of the resulting powder was mixed with 500 ml of distilled water and continuously agitated for 24 hours at room temperature; then the mixture was filtered by Whatman paper No. 3.

2- Extraction by organic solvents

Successive maceration by three solvents of increasing polarity (petroleum ether, chloroform and methanol) was used as extraction method according to Lavanya et al. [18]. 50 g of the powder was extracted with 250 ml of petroleum ether and placed under mechanical agitation for 24 h at room temperature. After filtration on Whatman paper, the residue from the previous extraction was taken up by 250 ml of chloroform

and left in agitation for 24 h. The residue is re-extracted per 250 ml of methanol for 24 h under the same conditions. Each extraction step is repeated three times with solvent renewal. All three extracts were vacuum-concentrated on rotavapor at 40°C. The three extracts of each solvent were pooled and vacuum-concentrated on rotavapor at 40°C. The four obtained extracts were considered as the stock solution and a dilution series (125 µg/ml, 250 µg/ml, 500 µg/ml, 750 µg/ml, and 1000 µg/ml) was prepared for each.

Phytochemical screening

The chemical characterization tests covered the investigation of the different chemical compounds in all different extracts. These characterizations were made using mainly tube reactions. The results are classified into:

- Very positive reaction +++
- Positive reaction +
- Negative reaction –

1- Alkaloid test

Precipitation reaction was adapted for the detection of the alkaloids using Dragendorff reagent [19]. 5ml of the extract was introduced in test tubes, and then added 2 ml of HCl and 1 ml of Dragendorff reagent. The formation of a red or orange precipitate indicates the presence of alkaloids.

2- Flavonoid test

1ml of extract was added to NaOH. Let it work for 3 minutes. An intense yellow coloring indicates the presence or not of flavonoids [20].

3- Tannin test

The addition of 2% iron trichloride (FeCl_3) to the test tube with 2 ml of extract can detect the presence or the absence of tannins. The color turns black brown in the presence of gallic tannins (hydrolysable tannins) and heartworm blue in the presence of catechic tannins (condensed tannins) [21].

4- Steroid test

Dissolve the extracts in 1ml of chloroform and add 1 ml of concentrated sulfuric acid. A positive test is revealed by the appearance of a red top layer [21].

5- Reducing compounds

Their detection consists in treating 1ml of the extract with 2 ml of distilled water and 20 drops of Fehling's solution, then heating. A positive test is revealed by the formation of a red-brick precipitate [22].

6- Saponins test

Mix 1ml of the extract with a few drops of distilled water and then shake strongly the solution. The mixture is left for 15 min. The foam persistence of at least 1 cm indicates the presence of saponins [23].

7- Terpenoids test

Dissolve the extracts in 1 ml of methanol and add 2 ml of CHCl_3 then 1ml of acetic anhydride and 1ml of sulfuric acid. Positive detection is revealed by violet staining [24].

Hemolytic activity

The hemolytic activity was evaluated according to the method described by Sanjeeb in 2011 [1].

Preparation of erythrocyte cells

Blood samples were collected from healthy volunteer donors of the same blood group and aged 22 to 25 years by vein puncture in heparin tubes. 5 ml of the blood was centrifuged for 3 minutes at 1500 rpm; the obtained pellet was washed 3 times with a sterile saline phosphate buffer solution (pH=7.2 0.2) by centrifugation for 5 min at 1500 rpm. The cell suspension was returned to a normal saline solution at 0.5%.

Hemolytic activity test

0.5 ml of each extract at different concentrations (125, 250, 500, 750, and 1000 $\mu\text{g/ml}$ in the saline phosphate buffer) was mixed with 0.5 ml of the erythrocyte suspension obtained previously. The mixture was incubated for 30 minutes at 37°C and centrifuged for 10 minutes at 1500 rpm. The free hemoglobin in the supernatant was measured using the UV-Vis spectrophotometer at 520 nm. The saline phosphate buffer is used as the minimum control of activity, while the distilled water is used as the maximum control. Each experiment was performed three times. The percentage of hemolysis is calculated by the following formula:

$$\% \text{ Hemolysis} = [(At - An) / (Ac - An)] \times 100,$$

Where **At**: absorbance of the test sample

An: minimal control absorbance (*phosphate buffered saline* PBS)

Ac: maximum control absorbance (distilled water)

Statistical analysis

The tests were performed in triplicate. The statistical analysis of one-criterion variance was performed by the SPSS software (Statistical Package for the Social Sciences) version 19.

Results and Discussion

Phytochemical screening helps to reveal the chemical nature of the constituents of the different plant extracts; it can be used to search for bioactive substances used in the synthesis of some drugs [25]. The results of the phytochemical tests carried out on the aqueous, methanolic, chloroformic and petroleum ether extracts of *L. camara* leaves revealed the presence of a variety of phytochemical compounds such as phenolic compounds, saponins, sterols, tannins and flavonoids as a major compound; reducing compounds as minor compounds; and the absence of alkaloids in the four extracts (Table 1).

Table 1. Results of phytochemical tests on different extracts of *L. camara* leaves

Extract Compounds	Aqueous extract	Methanolic extract	Chloroform extract	Petroleum Ether extract
Alcaloids	-	-	-	-
Sterols	+	+	-	+
Saponin	++	+	+++	+++
Phenolic compound	+++	+++	+++	+++
Reducing compound	-	-	-	+
Gallic tannins	+++	-	+++	-
Catechic Tannins	-	+++	-	+++
Terpenoids	-	-	-	-
Flavonoids	-	-	+++	+++

The hemolytic activity of chloroformic, petroleum ether, methanolic and aqueous extracts of *L. camara* leaves was tested on human erythrocytes and expressed as percentage of hemolysis. The obtained results (Figure 1) showed that the tested extracts have medium hemolytic effect. The methanolic extract at the concentration of 1000 µg/ml induces the lowest hemolytic activity: 23.2%, while the chloroformic extract at the same concentration has the highest hemolytic activity: 39%. The same results also showed that the hemolytic effect depends on the concentration of extract. The hemolytic effect of the different test extracts can be classified as follows: Chloroformic extract > Petroleum ether extract > Aqueous extract > Methanolic extract. The one-criterion variance analysis showed a very highly significant difference ($P \leq 0,0001$) between the four extracts in the different concentrations.

The value of medicinal plants lies in their richness in phytochemical constituents such as flavonoids, phenolic compounds, tannins, terpenoids, sesquiterpenes, etc., which cause a definitive pharmacological action on the human body. The results obtained in this study are similar to those of Oyedara [25], Naeem [26] and are somewhat similar to those of Jo-Ann T. Salada [27] on the essential oils of the leaves of *L. camara* and those obtained with Tripathi [28].

The hemolytic activity of any compound is an indicator of general cytotoxicity to normal healthy cells [29]. The medium hemolytic effect of the four extracts indicates their medium cytotoxicity to human

erythrocytes [1]. This test is useful in determining whether cytotoxic activity is related to direct damage to the membrane or not. Red blood cells are among the most commonly used cells in the toxicity assessment because of their availability, and the ease of their monitoring during cell lysis through the release of hemoglobin. These globules were used as a good cell model to assess the cytotoxicity of different molecules, particularly those isolated from medicinal plants, in order to determine the toxicity of these plants [30].

Figure 1. Hemolysis percentage of different extracts of *Lantana camara* according to their concentrations

Several natural and synthetic organic compounds are capable of destabilizing the membrane of red blood cells and lead to their lysis. Peptides rich in cysteine and saponins isolated from plants have very interesting biological activities but they also have hemolytic power. Saponin is a major component of *L. camara* which acts as a secondary metabolite modifying the surface tension of the extracellular medium [31, 32]. Since saponins are terpenic components, the hemolytic effect of this plant can be explained by the presence of these molecules, which have the ability to induce pore formation through cell membranes, resulting in haemolysis and release of hemoglobin into plasma [33].

The hemolytic activity of saponins is considered the result of affinity of the aglycone fraction of these molecules to membrane sterols, especially cholesterol by forming an insoluble complex (saponin-cholesterol micelles), which leads to the destabilization of the lipid bilayer with permanent pore formation in the membrane, and hence to cell lysis [34]. Medicinal plants, despite their therapeutic effects, should be used with the utmost caution as they may have a risk of toxicity [35].

Conclusion

Medicinal plants play a very important role in the medicinal field. Among these plants *L. camara* which has long been used in traditional medicine to treat various diseases. In this study, we reported the phytochemical composition of four extracts of *L. camara* leaves as well as their hemolytic activity. The results obtained showed the richness of this plant in phytochemicals and its ability to exercise a medium hemolytic activity which reflects its cytotoxicity to normal healthy cells. As perspectives, it is suggested to further study this plant by extraction of bioactive molecules which will be used in the production of herbal medicines; and to deepen the trials by studying other activities such as anticoagulant activity and further study its toxicity *in vitro* and *in vivo*.

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