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Phytochemical screening and antibacterial activity of aqueous extracts of *citrullus colocynthis* seeds

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Abstract- Skin, gynecological and lung infections are caused by microorganisms exist in the world. The treatment of these infections is mainly based on the use of synthetic drugs that become, in recent years, ineffective, due to the resistance of bacterial strains and the proliferation of opportunistic fungi. The aim of this study is to test in vitro the antibacterial activity of aqueous extract from seeds of *Citrullus colocynthis* which was detected phytochemical families existing. These antimicrobial powers are measured using the micro-dilution method against the Gram-negative bacteria (Escherichia coli ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) and Gram-positive (*Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923). The results showed that seeds of *C. colocynthis* are rich by bioactive substances. All tested extracts of *C. colocynthis* showed antimicrobial activity against all strains tested.. The folk medicinal use as a broad-spectrum antimicrobial agent is validated.

Key words: *Citrullus colocynthis Schrad* ; Phytochemical family ; Antibacterial activity.

INTRODUCTION

In some underdeveloped country, plants are the main source of drugs to treat infectious diseases due to the availability and the economic climate. However, only about 20% of plants were subjected to pharmacological studies or laboratory tests, despite the large number of new derivatives of natural resources or semi-synthetic is introduced on the market [1] antibiotics. The natural substances from plants have multiple interests put to use in industry, for food, cosmetics and pharmacology. Among these compounds are found largely secondary metabolites which are mainly used in therapy.

The pharmacy still uses a high proportion of plant and research is directed towards the discovery of new bioactive molecules drugs or raw materials for the semi synthesis [2]. *Citrullus colocynthis* Schrad. (Cucurbitaceae) grows increasingly in drylands is an endemic plant in southern Algeria[3].

This herb is widely used in traditional Algerian medicine for the treatment of many diseases such as arthritis, hypertension, and various infectious diseases, including dermatological and

gynecological problems, urinary and pulmonary infections [4, 5, 6]. It has other therapeutic properties purgative, anti-tumor and immunostimulatory [7, 8], anti-inflammatory and antioxidant [9, 10], antirheumatic [11], laxative and against leukemia, jaundice, fever, ascites, biliary disorders and hemorrhoids. [12] It is also used against liver disease [13]. The ethanol extract of the fruit of bitter gourd has an anti-microbial effect on Pseudomonas aeruginosa and Staphylococcus epidermidis and antifungal effect of several types of fungi [14]. The present study aims to investigate in vitro antibacterial activity of the seeds of this plant using a series of micro-broth dilution after extraction and phytochemical study

MATERIEL AND METHODS

Fruit of *Citrullus colocynthis*

Matured fruits of Citrullus colocynthis was taken from the region of Oued N'sa located about 70 km from the town of Ouargla during the month of December 2010. The identification of plant species is performed at the Laboratory of Plant Physiology at the University of Saida by Dr. Hasnaoui on the support of his experience and some documentation regarding the taxonomy of this species in the plant kingdom [3].

Aqueous extraction

The aqueous extract was prepared by maceration in cold distilled water at 20% for three hours. The mixture is then centrifuged at 3600 g for 30 min. The supernatant was recovered and filtered through paper filter Whatman No. 01. The fraction obtained is collected in a vial and stored at 4 °C in the dark until their use. [15] In order to calculate the yield of extraction, the aqueous extract is recovered by evaporation of water in an oven at 50 °C.

Phytochemical screening

The extract was subjected to phytochemical screening to identify existing phytochemical families. The tannins are detected by the method of Karumi et al. [16] Coumarins, steroids and anthocyanins were found using the technique of Bruneton [17]. Flavonoids have been detected by the method of Malec and Pamelio [18], anthraquinones by the method of Oloyede [19], the saponins by the method of Koffi et al [20] and finally the alkaloids are revealed by the method of Mojab et al [21].

Antibacterial activity and determination of MIC and MBC

Four reference strains were selected for antibacterial investigation: Gram-positive cocci (*Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923) and Gram-negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853).
The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were measured by the method of micro-broth dilution using 96-well microplates, following the procedure of Berche et al. [22]. The extract solutions were prepared by dissolving in 10% dimethyl sulfoxide (DMSO). The extract concentrations tested ranged from 0.003 to 1.600 mg / ml. The (MIC) of each aqueous extract was defined as the lowest concentration that inhibits bacterial growth after incubation at 37 ° C between 18 and 24 h. The minimum bactericidal concentration (MBC) was determined by subculture on blood agar at 37 ° C between 18 and 24 h. Levofloxacin was used as antibacterial positive control.

RESULTS

Extraction yields and phytochemical study

The calculation of the yield relative to the total weight of the dry powder of *C. colocynthis* seeds used in aqueous extraction shows that the plant gave masses greater than 1 g/100 g seed powder.

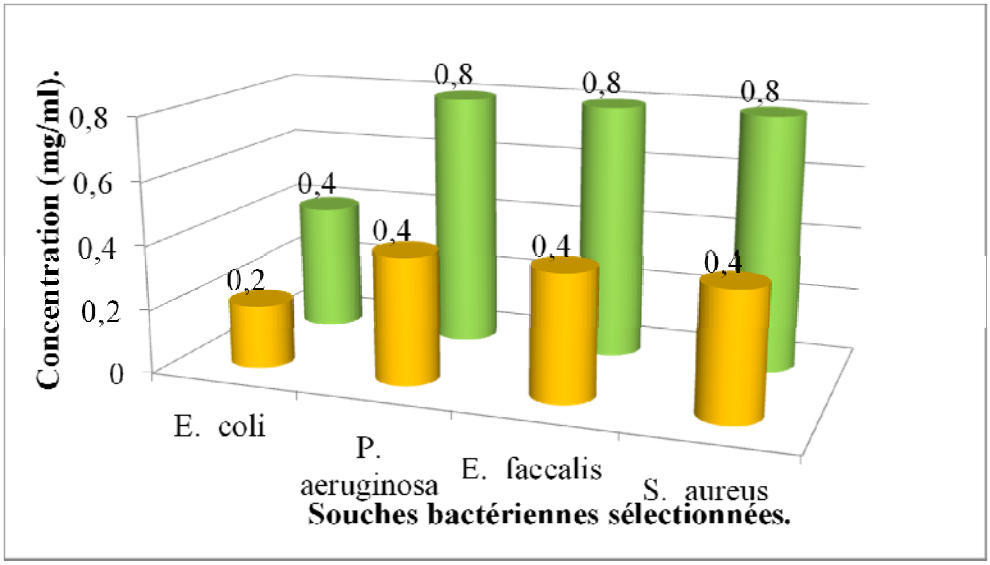
Table 1. Extraction yield and phytochemical study of the aqueous extract of *C. colocynthis* seeds.

Extracts types	Extraction yields	Polyphenols					Saponosids	Terpens Steroids	Alka loids
		Tannins	Anthraquinons	Flavonoids	Anthocyans	Coumarins			
Aqueous extract	1.83 %	+	-	+	+	-	+	+	+

Chemical characterization of the aqueous extract showed the presence of polyphenols in the form of hydrolysable tannins, flavonoids, saponins and anthocyns while anthraquinoes and coumarins are entirely absent. The same extract reacted positively vis-à-vis the chemical test seeking steroids (belonging to the group of terpenes) and alkaloids (Table 1).

Antibacterial activity

The aqueous extracts of C. colocynthis seeds showed a good antibacterial activity against four strains selected (Figure 1).



***CMI en jaune et CMB en vert**

Figure 1. Determination of MIC and MBC of the aqueous extract of *C. colocynthis* seeds on different bacterial strains selected.

The concentration range selected for the determined MIC and MBC varies between 0.003 to 1.6 mg/ml. The highest inhibitory activity was obtained against *Echirichia coli*, the MIC was 0.2 mg/ml while the CMB is 0.4 mg/ml. For the three other bacterial strains, the MIC was 0.4 mg/ml, while the CMB is 0.8 mg/ml (Figure 1).

DISCUSSION

Medicinal plants have long been used for the treatment of various diseases. Currently, they are used for the treatment of difficult infections to treat by drugs. The antimicrobial

activity of the organs of *C. colocynthis* is elucidated in several studies [6, 10, 14]. For cons, the phytochemical and antimicrobial screening of the aqueous extract of C. colocynthis seeds from the region of Ouargla is not clarified.

This study yielded several results such as aqueous extraction yield, the phytochemical study and antimicrobial activity. The extraction yield depends on the solvent and the same method by which the extraction is performed, and the effectiveness of the extract depends on the type of extraction solvent, active substances and other non-active therefore incapable to made desired biological activity. The effectiveness also depends on the selected bacterial strains and plant organ tested. For bacterial strains, antimicrobial activity may be different from one bacterium Gram- to another Gram+ [23].

The good MIC of the aqueous extract of *C. colocynthis* seeds recorded against *Pseudomonas aeruginosa* is a very good result, because this bacteria is among the most common causes of nosocomial infections [24]. This reveals a new confirmation of antibacterial properties of *C. colocynthis* seeds. The recorded as antibacterial activity against *Escherichia coli* class *C. colocynthis* seeds among the most important medicinal plants used against digestive problems caused by this bacteria [23].

The results give an indication that the plant is producing certain chemicals and compounds toxic to microorganisms. Furthermore, the literature has shown that plant extracts have great potential inhibitor against resistant bacterial strains [25, 26].

CONCLUSION

The results presented in this study indicate that the analyzed natural products are a great choice for the development of new treatment strategy for gastrointestinal and pulmonary dermatological, gynecological infections. The use of this plant in traditional medicine for the treatment of infections is confirmed by the results obtained in this study. This plant can also be used as an alternative to other chemical formula in order to increase their activity and effectiveness. In another study will be made to purify and identify the chemical compounds of this antimicrobial extract

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