

Antibacterial activity of Saponins extract from *Lepidium aucheri boiss*

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Abstract

Saponins extract from *Lepidium aucheri boiss* was isolated and evaluated for antibacterial activity against gram negative and gram positive bacteria such as *E.coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Enterococcus faecalis*. The inhibitory effect in vitro was defined to appear inhibition zone around the well. The study revealed that 75% (w/v) concentration was most activity against these pathogenic bacteria.

Keywords: Extraction, *L.aucheri boiss*, Antibacterial activity

Introduction

Applications of medicinal plants are known to be used as food preservative due to its antimicrobial activity and antioxidant (1). Natural products have been a major source of new drugs(2). Researches on natural products have significantly progressed over the last decades, mainly on plants corroborating their importance to the discovery of new biological and medicinal agents (3, 4, 5). Medicinal plants are a source for a wide variety of natural products, such as saponins and phenolic acids which are very interesting for their antioxidant properties (6). *Lepidium aucheri boiss* height ranges between around me (2-15 cm), with white petals spread in different of Iraq, especially in the desert areas in the addition to the area of the alluvial plain. This is due to the type genus *lepidium* L. Which comprises several types in Iraq including *L.aucheri Boiss*. It is one kind of family Cruciferae. Image (1) explained the *L. aucheri Boiss* (7). The taking conservation and use of medicinal plants. Saponins are steroid and triterpenoid glycosides common in a large number of plants and plants product that play an important role in human and animal nutrition. Several biological effect have been ascribed to saponins (8, 9) and they are also believed to form the main constituents of many plant drugs and folk medicines and are considered responsible for numerous pharmacology properties (10). Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid or steroid in nature (11, 12). Many saponins have detergent properties. They lower the surface tension of aqueous solution and therefore give stable foams when in contact with water. The aim of the present study was to evaluate

antibacterial activity of saponin extract from *L.aucheri Boiss* against bacterial pathogens.



Image (1): *Lepidium aucheri Boiss*

Material and method

Plants Collection:

Lepidium aucheri boiss was collected in March 2013 from north - west of Nasiriyah city at Iraq , then it was authenticated and specimen of plant was classified in biological department-college of science at university oh Thi - Qar in Iraq by Asst. prof. Hyder Radhi . The plant were cleaned , washed by distilled water , dried at room temperature for weeks and dried under the shade , ground as powder and kept in dark glass containers for further use.

Extraction of crude saponin from *Lepidium aucheri boiss*

Air-dried powdered leaves of *lepidium aucheri boiss* (100 g) was extracted with 70% ethanol (EtOH) (24h x 1000 mL) at room temperature the EtOH solution was concentrate to a small volume (300 ml) by low pressure evaporation at 45 °C and extracted in succession with chloroform (24h x 100 ml x 3) and n-Butanol (24h x 100 ml x 3). The n-Butanol layer was concentrated to dryness given the saponins extract (10g) (13,14).

Test of saponins compounds

(1) Aqueous solution of saponin extract has prepared in test tube and was shaken after that product soapsuds was remaining and stable.

(2) 5 ml from aqueous solution of saponin extract in test tube and added 5 ml from 5% silver nitrate solution after that put in waterbath at 100 °C for 5 minutes when test tube cooled produced silver mirror on inner surface of the test tube (15).

Antibacterial assay

Culture Media:

Muller Hinton Agar was supplied from Himedia company (india), it was used as a culture medium and was prepared depending on information determining by manufacturing company .

Pathogenic Bacterial Strains

Staphylococcus aureus and *Strep coccus* and *Enterococcus faecalis* (positive to Gram stain), *Escherichia coli* and *Pseudomonas aeruginosa* (negative to Gram stain) were used as pathogenic bacteria strains and they were identified in biology dept. college of Science in Thi- Qar University.

Assessment of Antibacterial Activity of Saponins Extracts

Bacteria suspension of each tested bacteria (10⁷ CFU/mL) was spread onto the surface of Muller-Hinton agar plates. Eight mm cork borer was used to punch wells into the plates and 100 µL of each extract dissolved in DMSO (50% and 75% w/v) as well as were applied to each well. The plates were incubated for 18 h at 37°C. The inhibition zones diameter for each concentration was measured and the saponins coefficient was calculated (16).

Results and Discussion

The result of test saponins in crude extracts from *lepidium aucheri boiss* gave stable foams in water for long time (more than one hour) and the second test procedure silver mirror on inner surface of the test tube table (1) . Saponins have shown activity against a broad range of microorganisms including bacteria, filamentous fungi and yeasts(17, 18). The result of antibacterial activity are presented in table (1) which summarize the inhibition zone around the well measured by diameter. The concentration was 75% (w/v) more effective inhibition zone of Gram positive like *Staphaureus* (22mm), *Streptococcus* (20mm), also Gram negative bacteria like *Pseudomonas* (15mm) table (2) . This results are similar to (19) with (20). We can use this study for development new antibacterial for need when some bacteria pathogen resistant to available antibiotic agents. *Lepidium aucheri boiss* plant more distribution in south and middle of Iraq and used in folk medicine, can help for make when new antibacterial cheap cost. When can isolation compounds of saponin from crude after that found which compound has highly biological

activity, this is extract broad spectrum activity on both gram negative and gram positive.

Table (1): Qualitative tests of saponin extract in *lepidium aucheri boiss*

Active principle	Test	Saponins Extract
Saponins	shaking	+
	Silver nitrate	+

Table (2): Diameters of inhibition zone (mm) for all extracts

Type of Bacteria	Zone of inhibition in mm			
	Gram stain	50% of Saponins Extracts	75% of Saponins Extracts	Control (Ciprofloxacin)
<i>E.coli</i>	-	7	13	17
<i>Pseudomonas</i>	-	10	15	20
<i>Staphaureus</i>	+	16	22	24
<i>Strep coccus</i>	+	13	20	22
<i>Enterococcus faecalis</i>	+	10	14	16
DMSO		0	0	

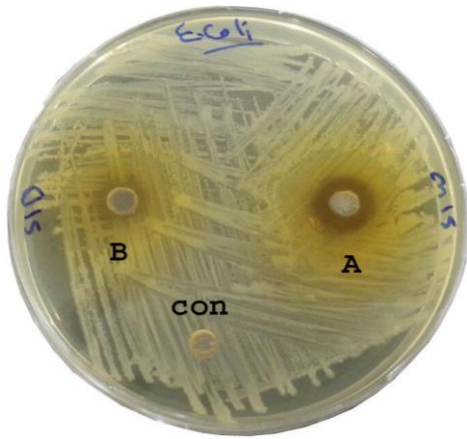


Figure (1): The activity of Saponins extracts against (*E.Coli*) bacteria
1: A 75% Saponins extract
2: B 50% Saponins extract Con: (DMSO)



Figure (2): The activity of Ciprofloxacin against (*E.Coli*) bacteria
E.Coli: Escherichia coli

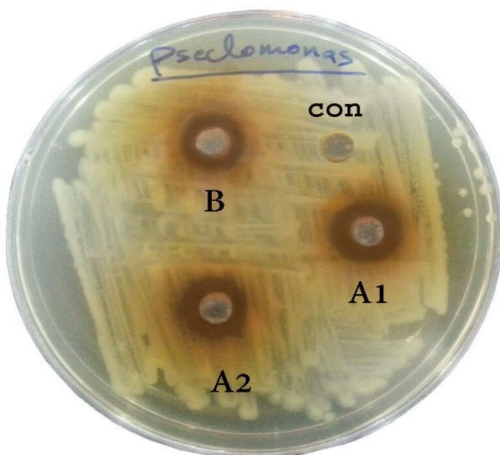


Figure (3): The activity of Saponins extracts against (*Pseudo.*) bacteria



Figure (4): The activity of Ciprofloxacin against (*Pseudo.*) bacteria
Pseudo: Pseudomonas aeruginosa

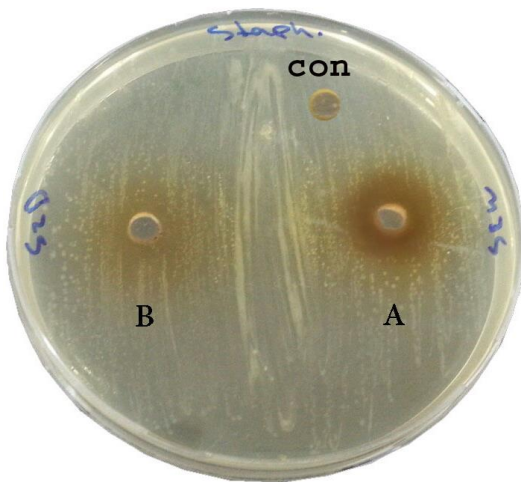


Figure (5): The activity of Saponins extracts against (*Staph.*) bacteria.



Figure (6): The activity of Ciprodar against (*Staph.*) bacteria
Staph: Staphylococcus aureus.

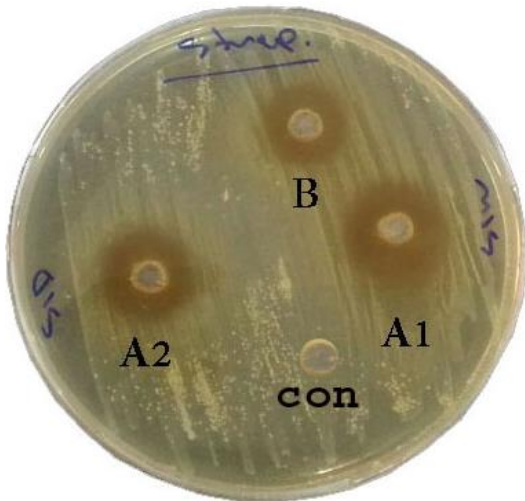


Figure (7):The activity of Saponins extracts against (*Strep.*) bacteria



Figure (8): The activity of Ciprodar against (*Strep.*) bacteria
Strep. : Strepto coccus

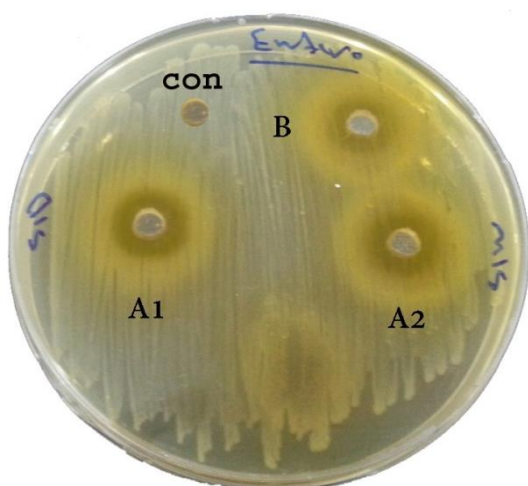


Figure (9):The activity of Saponins extracts against (*Enterococcus*) bacteria



Figure (10):The activity of Ciprodar against (*Enterococcus*) bacteria
Enterococcus : *Enterococcus*

References:-

- 1- Sunilson, J. A. J. R., Suraj, G., Rejitha, K., Anandarajagopal, A. V. A. G., Kumari and P. Promwicht. (2009). *In vitro* antimicrobial evaluation of *Zingiber officinale*, *Curcuma longa* and *Alpinia galangal* extracts as natural food preservatives. *Am. J. Food Technol.* 4: 192-200.
- 2- Vuorela, P., Leinonen, M., Saikku, P., Tammela, P., Wennberg, T. and Vuorela, H. (2004). Natural products in the process of finding new drug candidates.. *Current Med. Chem.* 11: 1375-1389.
- 3- Calixto, J. B. (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Braz. J. Med. Biol. Res.* 33(2): 179-189.
- 4- Rates, S. M. K. (2001). Plants as source of drugs. *Toxicon.* 39: 603-613.
- 5- Newman, D. J., Cragg, G. M. and Snader, K. M. (2003). Natural products as sources of new drugs over the period 1981-2002. *J. Nat. Prod.* 66: 1022-1037.
- 6- Farhan, H., Rammal, H., Hijazi, A. and Badran B. (2012). Preliminary phytochemical screening and extraction of polyphenol from stems and leaves of a Lebanese plant *Malva Parviflora*. *Int J Curr Pharm Res.* 4(1): 55-59.
- 7- Townsend, C. C. and Guest, E. (1980). Flora of Iraq. *Minis. Agricu. Iraq.* 4: 2. 886.

- 8- Sparg, S. G., Light, M. E. and Van Staden, J. (2004). Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology* 94(2-3) : 219-243.
- 9- Lacaille-Dubois, M. A. (2005). Bioactive saponins with cancer related and immunomodulatory activity: recent developments. *Studies in Natural Products Chemistry* 32(12): 209-246.
- 10- Liu, J. and Henkel. T. (2002). Traditional Chinese medicine (TCM): are polyphenols and saponins the key ingredients triggering biological activities. *Current Medicinal Chemistry* 9: 1483-1485.
- 11- Oleszek, W. and Bialy, Z. (2006). Chromatographic determination of plant saponins an update (2002-2005). *Journal of Chromatography A* 1112(1-2): 78-91.
- 12- Bruneton, J. (2009). *Pharmacognosie-phytochimie, plantes medicinales*, 4eme edition, revue et augmente, Tec&Doc-Edition Medicinales Internationals Paris. 1288 p.
- 13- Ivan kostova. Dragomir, D., Gudrun, H.R., Vladimir, D., Antoaneta, I. (2002). Two new sulfated furostanol saponins from *Tribulus terrestris*. *Zeitschrift Naturforsch* 57c. 33-38.
- 14- Flavio, H.R., Carla, K., Jan, S., Dominique, G., Grace, G., Eloir, P.S. (2001). Steroidal and triterpenoidal glucosides from *Passiflora alata*. *J.Braz.Chem.Soc.vol.12(1)*: 32-36.
- 15- Shihata, I.M. (1951). A pharmacological study of *anagallis arvensis*, M.D. vet. Thesis. Cairo University.
- 16- Jeremiah, E. Angeh, Xueshi Huang, Gerry, E. Awan, Ute Mollman, Isabel Sattler and Jacobus N. Eloff. (2007). Novel antibacterial triterpenoid from *Combretum Padoides* . 113-120.
- 17- Lacaille-Dubois, M. A. and Wagner, H. (1996). A review of the biological and pharmacological activities of saponins. *Phytomedicine*. 4: 363-386.
- 18- Lacaille-Dubois, M. A. and Wagner, H. (1999). Bioactive saponins from plant: anupdate, in *atta-ur-Raman(ed.)*, *Studies in natural products chemistry of organic natural products* Springer, Wien, New York, 74, 1-196.
- 19- Husam M. Kredy. (2010). Antibacterial activity of saponins extract from *sider (Ziziphus spina-christi)*. Vol.6: 3-4.
- 20- Deboshree Biswas and Roymon, M. G. (2012). Validation of antibacterial activity of saponin against diarrheagenic *E.coli* isolated from leave and bark of *Acacia arabica*. *Journal of Phytology*. 4(3) : 21-23.

الفعالية ضد البكتريا لمستخلص الصابونين المعزول من نبات الرشاد البري

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الخلاصة:

المركبات الصابونينية المعزولة من نبات الرشاد البري تمتلك تأثير مضاد للبكتريا السالبة لصبغة غرام مثل *E.coli* , *Pseudomonas aeruginosa* ولها تأثير مضاد للبكتريا الموجبة للصبغة مثل *Staphylococcus aureus* , *Streptococcus pneumoniae* *Enterococcus*, ظهر مختبريا يعرف بظهور منطقة التثبيط حول الحفرة وقد أظهرت الدراسة أن تركيز 75% (w/v) كان التركيز المثبط الأعلى .

الكلمات المفتاحية: الاستخلاص, نبات الرشاد البري, الفعالية ضد البكتريا