

Biological Activity of Saponins Isolated from *Tribulus terrestris* (Fruit) on Growth of Some Bacteria

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Abstract:

In this study, the saponin compounds were isolated from *Tribulus terrestris* (Fruit), and to determine the inhibitory effect of these compounds were investigated on the growth of some bacteria, which include: *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, *Klebsiella pneumonia* and *Bacillus subtilis*, and used the antibiotics (Ciprofloxacin, Chloramphenicol) as control samples. The saponin compounds were further analyzed by IR and identified by thin layer chromatography (TLC plate). The saponin compounds showed significant inhibiting effect on the all bacteria which was used in this study compared to commercial antibiotics as a control.

Key words: biological activity, saponins, *Tribulus terrestris*.

Introduction:

Plants and plant products have been used extensively throughout history treat medical problems. Numerous studies have been carried out to extract various natural products for screening antimicrobial activity [1]. *Tribulus terrestris* L. [Zygophyllaceae] is annual herb of worldwide distribution. The plant is used in the folk medicine in India, China, Bulgaria and other countries against various diseases [2,3,4]. It is known as a goathead in the USA ying-jili in China and dze-mara-goma in Bhutan. The genus *Tribulus* comprises of approximately 20 species which grow as shrubs about 10-60 cm high [5,6].

The fruits are light brown when mature and measure about a centimeter in diameter. It has sharp spines (2 spines measuring 2-3 cm) that are accompanied by several spikes around it. The fruits and seeds are of great importance to traditional or natural medicines that are practiced worldwide [7].

Tribulus terrestris is widely used as a component of therapy for a number of conditions, especially in China. It is known to treat conditions affecting the liver and kidney as well as the cardiac vascular and immune systems [8] and in Bukgaria *Tribulus terrestris* increases levels of various hormones in the steroid family, including testosterone, DHEA and estrogen [9,10].

The plant has been extensively investigated by a number of works, the presence of steroidal glycosides, steroidal, saponins, flavonoids and alkaloids [11]. Saponins such as glucopyranosyl, galactopyrans, ruscogin, hecogenin, gitogenin, titogenin, protodioscin, diosgenin and yamogenin [12,13].

In this present work, *Tribulus terrestris* (fruits) was used to get some biological active saponin compounds and test its biological activity on growth of some Gram positive and negative bacteria.

Material And Methods:

Plant Materials:

Tribulus terrestris (fruits) was a crop of 2005, obtained as semi-dry fruit from Mosul City in the Northern part of Iraq. The taxonomic identification of plant materials was confirmed by Department of Biology, College of Science, University of Mosul. The plant was dried and powder at room temperature for three weeks.

Extraction and Isolation:

Powdered plant material (500 g) was extracted with 70% ethanol (3 × 24 h) at room temperature. The combined

ethanol solutions were concentrated to a small volume and extracted in succession with chloroform (3 × 24 h) and n-BuOH (3 × 24 h). The n-BuOH layer was concentrated to dryness to give crude saponin extract (2 g) [14].

Thin Layer Chromatographic on Silica Gel:

For analysis of the saponin, thin-layer chromatographic was used in silica gel, and with the following solvent system a chloroform-methanol-water (80:20:2) which were detected after spraying with vanillin/ sulphuric reagent [15].

Saponin Analysis:

Saponin was using IR, using liquid film, solid KBr disc, and Infrared Spectrometer Model Tensor 27 Bruker Co., Germany [16].

Micro-Organisms Tested:

The following strains of bacteria were used:

Escherichia coli
Salmonella typhi
Klebsiella pneumonia
Staphylococcus aureus
Bacillus subtilis

They were obtained from department of Biology / College of Education / Mosul University.

Choosing the Effective Concentration:

The choice of effective concentration was carried by preparing stock solution of the extract in dimethyl sulfoxide (DMSO) (1:5/w:v) 200 mg/ml and then diluted it to (100, 50, 25, 12.5 mg/ml) to be applied in the study [17].

Antibacterial assay:

Leven et al. (1997) [18] method that depended on Vandepitte et al. (1991) [19] method was followed to perform this test.

Nutrient agar was incubated by using single colony of the five types of bacteria a foresaid singly, then the media was incubated at 37 °C for 24 hrs. The microbial suspension was diluted by normal saline solution by comparison with standard test tube (Macferland No. 1). It contained 10⁸ cell/cm³ from the microbial suspension. Then it was spread on agar media surface by using glass spreader, the dishes were incubated for 30 minutes until the absorption has been completed. Then, the dishes were prepared from filter paper (Whatman No. 1) diameter (6

mm), and saturated by different concentrations of isolated material from plant under test.

The disks were fixed by sterilized tong and incubated at 37 °C for 24 hrs. and finally the inhibiting regions were measured and compared with standard antibiotics positive control sample [20].

Results And Discussion:

The antimicrobial activity of saponin was extracted from *Tribulus terrestris* against the microorganisms examined in the present study, and the potency, were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and zone diameter. The results are given in Table (1).

Saponin extract showed different inhibiting effect on all bacteria, the best inhibiting effect was showed on *Staph. Aureus* and *E. coli*, its showed effect on *S. typhi*, *B. subtilis* and *K. pneumonia* greater than antibiotics (Chloramphenicol and Ciprofloxacin).

From the results above, the saponin was extracted from *Tribulus terrestris* that had a high inhibiting effect on bacteria growth.

The activity of the plant against both Gram-positive and Gram-negative bacteria was indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant [21]. The extract of this plant showed very strong antibacterial [22]. It was the

activity of the *Tribulus terrestris* extract which could be due to unknown steroidal saponin [23].

The saponins were studied by the infrared spectroscopy so as to know the structure of the isolated compounds. We can observe the main bands that appear on the Figure (1). They are: the stretching frequency band of hydroxyl groups (OH) at (3422 cm^{-1}), methyl groups and another alkyl groups at (2856 and 2928 cm^{-1}), aromatic(C-H) bands at (3010 cm^{-1}), carbonyl groups (C=O) at (1712 and 1744 cm^{-1}), aromatic system at (1465-1550 cm^{-1}), and finally (C=C) bond (weak) that appear at (1630 cm^{-1}). From all these bands that are clear by type or grade (strength and weakness of bands), we can say that compounds were isolated from the structures of saponins and their derivatives.

They consist of a fat-soluble nucleus, having either a steroid or triterpenoid structure. In the present study, the isolation of saponins and the study of the antimicrobial activity of these compounds were reported.

The physiological role of saponins in plants has not been fully understood yet. While there is a number of publications describing their identification in plants, and their multiple effects animal cells and on fungi and bacteria, and few studies have addressed the effect of saponin in plants on cells. Many saponins are known to be antimicrobial [24].

Table (1): Inhibiting activity of saponin from *Tribulus terrestris* (fruits) comparison with antibiotics

Conc.	<i>E. coli</i>	<i>S. typhi</i>	<i>K. pneumonia</i>	<i>S. aureus</i>	<i>B. subtilis</i>
200	24	23	14	26	20
100	22	20	10	23	17
50	18	19	8	17	13
25	12	16	-	14	9
12.5	9	10	-	12	-
Chloramphenicol	14	15	8	16	14
Ciprofloxacin	16	15	13	-	-

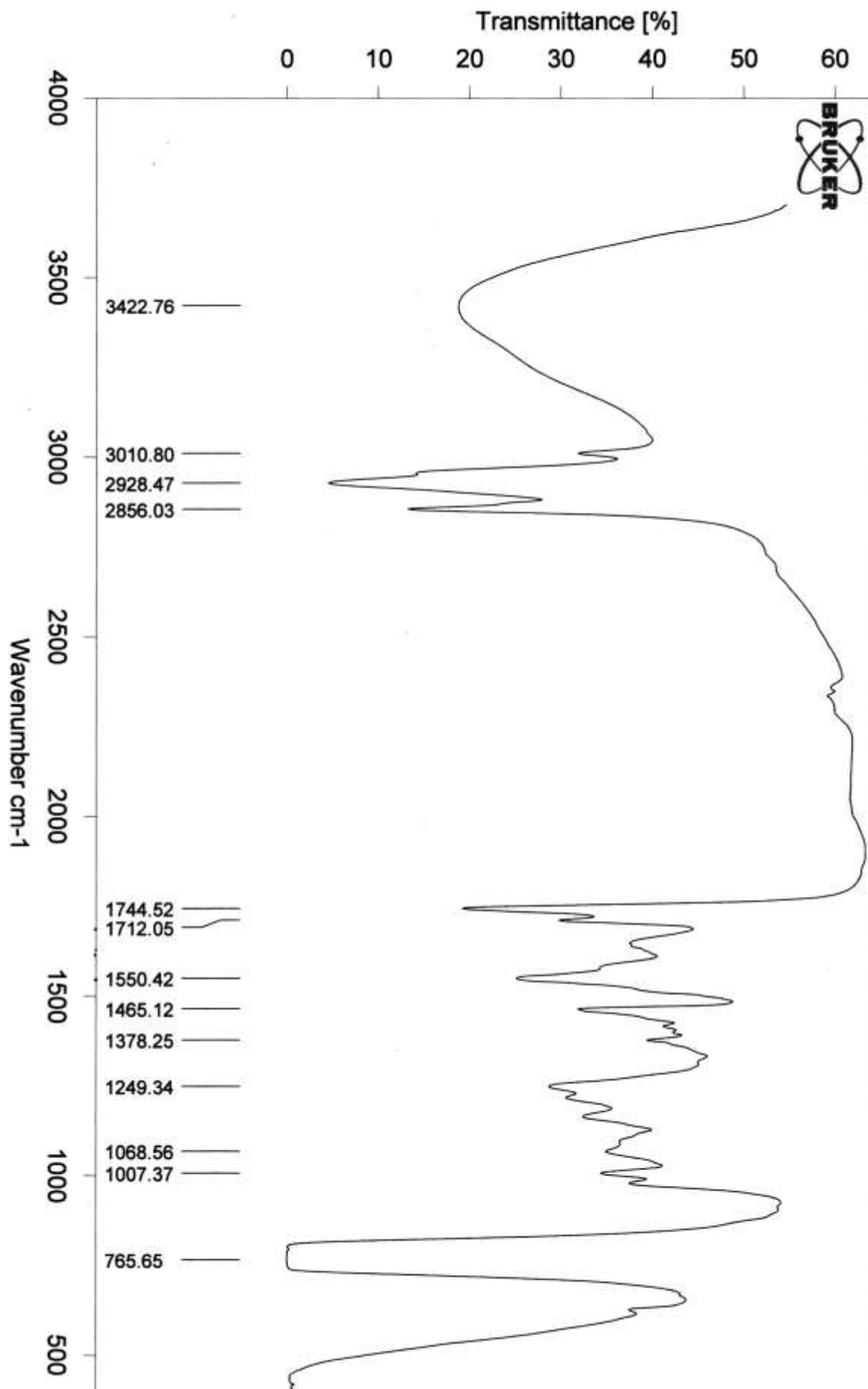


Figure (1): IR spectra of saponins

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الفعالية البايولوجية للصابونينات المعزول من ثمار نبات القطب *Tribulus terrestris*

في نمو بعض البكتريا

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الملخص:

تم في هذه الدراسة فصل مركبات الصابونينات saponins من ثمار نبات القطب *Tribulus terrestris*. وحدد التأثير التثبيطي لهذه المركبات في نمو خمسة انواع من الجراثيم هي *Escherichia coli* و *Salmonella typhi* و *Klebsiella pneumonia* و *Staphylococcus aureus* و *Bacillus subtilis*. واستخدم المضادين الحيويين (Chloramphenicol و Ciprofloxacin) كعينات سيطرة. وتم تشخيص مركبات الصابونينات المفصولة باستخدام طيف الاشعة تحت الحمراء (IR) كما استخدمت تقنية الطبقة الرقيقة في عملية التشخيص (Thin layer chromatography (TLC). اذ اظهرت مركبات الصابونينات فعالية تثبيطية عالية على جميع انواع الجراثيم المستخدمة قيد الدراسة مقارنة بعينات السيطرة.

الكلمات المفتاحية: الفعالية البايولوجية، الصابونينات نبات القطب