Biological Effect of Saponins Isolated from *Nigella sativa* (seeds) on Growth of Some Bacteria

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Abstract

This study is designed for isolation and identification of some saponin compounds from *nigella sativa*(seeds), and to determine the inhibitory effect of these compounds were investigated on the growth of some bacteria, which include: *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* and used the antibiotics (Gentamicin, Tetracycline) as control samples. The saponin compounds were further analyzed by IR and identificated 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, *nigella sativa*.

Itroduction

Medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency [1] *Nigella sativa* have been used traditionally for centuries in the Middle East, Northern Africa and South Asia for the treatment of various diseases [2] The plant is widely grown in different parts of the world. As an oriental spice, *Nigella sativa* has long been used as a natural medicine for the treatment of many acute as well as chronic conditions. [3] The seeds of *N. sativa* have been subjected to a range of pharmacological investigations in recent years. These studies have showed a wide spectrum of activities such as antibacterial, antitumor, anti-inflammatory, mutabagani and hypoglycemic, smooth muscles, relaxant, cytotoxic and immunostimulant [4].

Nigella sativa contain a yellowish volatile oil (0.5-1.6%), a fixed oil (35.6-41.6%), proteins (22.7%), aminoacids; e.g. albumin, globulin, lysine, leucine, isoleucine, valine, glycine, alanine, phenylalanine, arginine, asparagine, cystine, glutamic acid, aspartic acid, isoleucine, proline, serine, threonine, tryptophan and tyrosine [5] alkaloids, organic acids, tannins, resins, toxic, glycosidal saponins, [6] Several classes of compounds have been isolated from the seeds of *N. sativa*, such as alkaloids [7,8], flavonol triglycosides[9], saponins[10,11] and an isobenzofuranone derivative[12]. Saponins are steroid or triterpenoid glycosides, common in a large number of plants and plant products that are important in human and animal nutrition. Several biological effects have been ascribed to saponins[13] The aim of this study was to evaluate the antimicrobial potential of saponin compounds tested on a wide range of microorganisms.

Material And Methods

Plant Materials:

Nigella sativa (seeds) was obtained as dry from a local market, Mosul City. The taxonomic identification of plant materials was confirmed by Department of Biology, College of Science, University of Mosul[14].

Extraction and Isolation:

Powdered seeds of the plant (500 g) were defatted with *n*-hexane (2 x 3 L). Further extraction was performed with 80% MeOH (2x 6 L). The combined methanol 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[15].

Thin Layer Chromatographic on Silica Gel:

For analysis of the saponins, 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 [16].

Saponins Analysis:

Saponins was using IR, Infrared Spectrometer Model Tensor 27 Bruker Co., Germany [17].

Bacterial Culture:

In this study two types of Gram positive bacteria are used, they are: *Staphylococcus aureus* and *Bacillus subtilis* which were obtained from the department of Biology/College of Education/ Mosul University.

Another types of Gram negative bacteria *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus vulgaris* which were obtained from Veterinary College/Mosul university, while *Salmonella typhi* was obtained from department of Biology/College of Science/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[18].

Antibacterial assay:

Leven et al. (1997) [19] method that depended on Vandepitte et al. (1991) [20] 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 10^8 cell/cm^3 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[21].

Results And Discussion

In the present study, the antimicrobial saponin compounds from the *Nigella sativa* were extracted, and evaluated against awide range of microorganisms on the basis of disk diffusion and microdilution assays. antimicrobial activities were quantitatively assessed by the presence or absence of and inhibition zone and zone diameters (Table1). Our results showed that the saponin compounds has inhibitory effect on the growth of all bacterial species, namely *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Salmonella typhi*.

Saponin compounds showed different inhibiting effect on bacteria, higher effect was showed on *Staph. Aureus* ,*B subtilis* and *K. pneumonia*, and equal inhibiting effect was found on *S. typhi*, *and Pr. vulgaris*, so showed inhibiting effect best than antibiotics. it showed less inhibiting effect on *Ps. aeruginosa* compared with standard antibiotics (Gentamicin, Tetracycline).

The results are given in Table (1). From the results above, the saponin was extracted from *Tribulus terrestris* that had a high inhibiting effect on bacteria growth ,The development of resistant microorganisms on prolonged exposure to existing antimicrobial agents has been known for a long time[22]. There are also many reports of chemical investigations which have identified various chemical compounds responsible for the antimicrobial activities of ethnomedicinal plants [23] has led to the continual search for ways of eradicating resistant strains of micro organisms *Nigella sativa* extract and its constituents have been extensively studied for its their antimicrobial effect against a wide range of bacterial[24]. The compounds from the seeds of *N. sativa* in pure state and at various dilutions was screened *in vitro* against some microbes and it was found to exhibit promising activity against[25]. Finally, our results are in agreement with others who showed that *Nigella sativa* compounds produce antimicrobial activity against a broad range of microbes and especially on multiple antibiotic resistant bacteria[26].

The saponins were studied by the infrared spectroscopy so as to know the structure of the isolated compounds: The measurement of infrared spectroscopy of isolated saponin compounds showed the significant peaks in the regions of (1739)cm^{-1} (strong) related to (C=O) bond, (3337)cm^{-1} (weak) related to (-OH) group, (1243)cm^{-1} (medium) related to (C-O) bond, finally strong peak at (2855,2926)cm^{-1} related to methyl groups (-CH_3).

In this study, the most suitable TLC system for analysis was shown to be chloroform-methanol-water (80:20:2) with reagent (vanillin / sulphuric).

Thin-layer chromatography (TLC) is an ideal technique for the screening of drugs because of its low cost, easy maintenance and selectivity of detection reagents. TLC on silica gel is very favourable for the analysis of material plants[27,28].

Table (1): Inhibiting activity of saponin from *nigella sativa* (seeds) comparison with antibiotics (inhabiting diameter mm)

Conc.	*Staf.aureus*	*B.subtilis*	*Salm.typhi*	*K.pneumonia*	*Pr.vulgaris*	*PS.aeruginosa*
200	23	19	18	20	18	15
100	19	17	14	18	15	11
50	14	13	11	13	12	-
25	10	9	9	8	8	-
12.5	-	-	-	-	-	-
Gentamicin 10 mg/dick	14	-	14	9	15	12
Tetracyclin 30 mg/disk	23	-	23	14	18	22

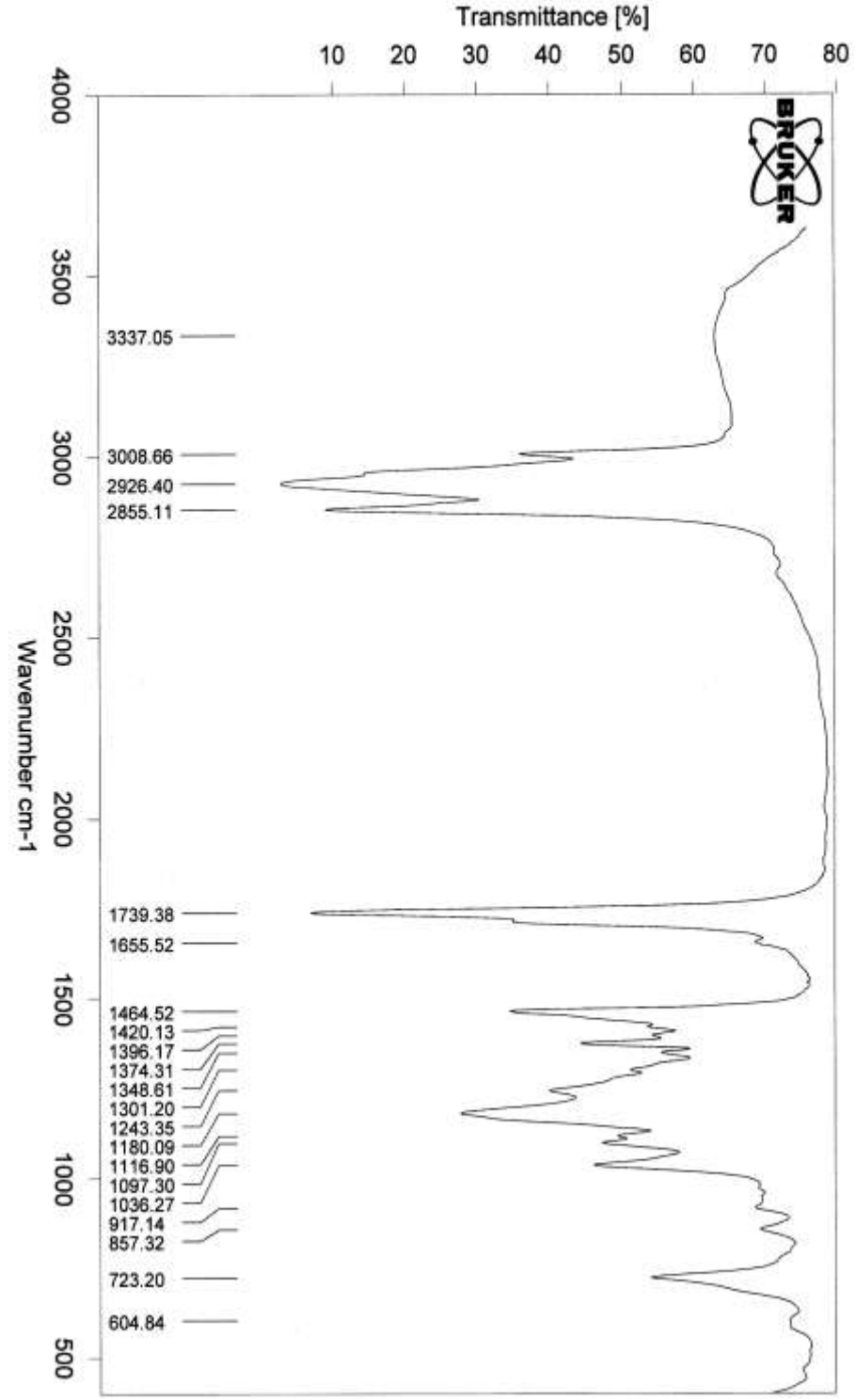

Figure (1): IR spectra of separated saponins from

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التاثير البايولوجي للصابونينات المفصولة من بذور حبة السوداء *Nigella sativa* في نمو بعض انواع البكتيريا

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الملخص:

تم في هذه الدراسة فصل وتشخيص بعض مركبات الصابونينات من ثمار نبات حبة السوداء *Nigella sativa* وتحديد التاثير التثبيطي لهذه المركبات في نمو عدد من الجراثيم: *Staphylococcus aureus* و *Bacillus subtilis* و *Pseudomonas aeruginosa* و *Klebsiella pneumonia* و *Proteus vulgaris* و *Salmonella typhi* . واستخدم المضادين Gentamicin و Tetracycline .

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.