

Effect of Aqueous and Ethanolic Extract of Aerial Parts of Parsley (*Petroselinum crispum*) on Some Bacteria Isolated from Nosocomial Infections *in-Vitro*.

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Abstract

Parsley (*Petroselinum crispum*) is important medicinal plant, has widespread consumption in food. The current study is aimed at declaration of antibacterial activity of aqueous and ethanolic extract of *P. crispum* aerial parts (stems and leaves) *in vitro* against five bacteria; *Shigella flexneri*, *Acenitobacter baumani*, *Morganella morgani*, *Enterococcus faecalis* and *Providencia* spp. isolated from different specimens of nosocomial infection. Aerial parts were extracted employing maceration method, antibacterial effect investigated by agar well diffusion method by following concentrations (0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 100, 150, 200 mg/ml) of each extract, compared with 10 µg/ml of ciprofloxacin and tetracycline as positive controls that were evaluated by agar disc diffusion method. Inhibition zones surrounding all extract concentrations and positive controls were measured. The influence of ethanolic extract exhibited zones of inhibition through (5-25) mm, while the inhibition zones resulted from the action of aqueous extract were between (5-27) mm generally. The extracts displayed perfect potency toward investigated bacteria as a whole. The aqueous extract was better than ethanolic extract on the strength of activity overall. In most cases, the effects to be based on rising the extracts concentrations. The 50 mg/ml concentration extracts was responsible for initiation the action as far as 200 mg/ml concentration altogether. The influence of maximum concentration (200 mg/ml) of the extracts was superior or likes to that of ciprofloxacin upon

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particular some bacteria. According to existing investigations it can be figuring out that the parsley aerial parts have effective antibacterial activity supporting their uses in traditional medicine, permitting their employment for treatment of nosocomial infections.

Key words: Parsley- *Petroselinum crispum*- Nosocomial infections.

تأثير المستخلص المائي و الايثانولي للاجزاء الهوائية للبقدونس (*Petroselinum crispum*) على بعض الجراثيم المعزولة من الاصابات الناجمة من المستشفيات في الزجاج.

ارجان ايدين زينل

قسم التقنيات التحليلات المرضية - الكلية التقنية/كركوك - الجامعة التقنية الشمالية - كركوك - العراق.

الخلاصة

يعتبر البقدونس (*Petroselinum crispum*) نبات طبي مهم، شائع الاستهلاك في الغذاء. استهدفت الدراسة الحالية تقييم فاعلية المضادة الجرثومية للمستخلص المائي و الايثانولي للاجزاء الهوائية (السيقان و الاوراق) للبقدونس في الزجاج ضد خمسة جراثيم: *Shigella flexneri*, *Acenitobacter baumani*, *Morganella morgani*, *Enterococcus faecalis* و *Providencia spp.* معزولة من نماذج مختلفة للاصابات التي نشأت في المستشفيات. تم استخلاص الاجزاء الهوائية باستخدام طريقة النقع. بحثت التأثير المضاد للجراثيم باستخدام طريقة الانتشار في حفر الهلام و بالتركيز التالية: (0,05، 0,1، 0,5، 1، 5، 10، 25، 50، 100، 150 و 200 ملغم/مل) لكل مستخلص و قورنت مع 10 مايكروغرام/مل من ciprofloxacin و tetracycline كمجموعة سيطرة موجبة التي قيمت باستخدام طريق الانتشار في قرص الهلام. و قد تم قياس مناطق التثبيط حول جميع تراكيز المستخلصات و مجاميع السيطرة الموجبة. اظهرت تأثير المستخلص الايثانولي مناطق تثبيط ما بين (5-25) ملمتر، بينما المناطق التثبيط التي نتجت من فعالية المستخلص المائي كانت ما بين (5-27) ملمتر عموماً. ابدت المستخلصات فعالية جيدة تجاه الجراثيم اجمالاً. المستخلص المائي كان افضل من المستخلص الايثانولي من حيث قوة الفاعلية ككل. في معظم الحالات كانت التأثيرات مستندة على اساس زيادة تراكيز المستخلصات. ان التركيز 50 ملغم/مل كانت مسؤولة عن بدء الفعالية وصولاً الى التركيز 200 ملغم/مل بالاجمال. ان تأثير التركيز النهائي (200 ملغم/مل) للمستخلصات كانت اعلى او مماثلة للذي سببها ciprofloxacin بخصوص بعض الجراثيم. وفقاً للدراسة الحالية يمكن الاستنتاج ان الاجزاء الهوائية للبقدونس لها فاعلية مضادة للجراثيم مؤثرة تؤيد استخدامها في الطب الشعبي و تجعله من الممكن استخدامها في علاج الاصابات الناجمة من المستشفيات.

الكلمات المفتاحية: بقدونس - اصابات الناجمة من المستشفيات.

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Introduction

Nosocomial infections (NI) are the infections which progress at the time of hospitalization and were not incubating or present during admission to the hospital (1). NI subsists a main threatening in high risk patients' with regard to increasing incidence of infection morbidity and mortality which can be decreased by applying by several control strategies (2). Bacteria isolation and description from suitable assayed materials with antimicrobial susceptibility testing is the criterion of laboratory diagnosis of NI (3). Multiple-drug resistant (MDR) bacteria greatly associated with NI (4).

It is fairly well recognized that MDR bacteria is a chief problem in the application of medicine and in public health (5). One of the alternative approaches to address the resistance threat includes new methods of antibacterial medication identification (6).

Natural products have an essential property in protection the human from diseases due to their safety without side effects, efficacy can be obtained over massive doses and they are more affordable (7). Herbal or traditional medicine forms the fundamental of natural products. Human used it since the ancient periods of history until the present time over the years throughout the world for treatment and prevention of sicknesses (8). Plant cells constitute miscellaneous chemicals, chiefly secondary metabolites for defense mechanism against bacteria, providing alternative medical treatment (9).

Various studies of plant solvent extracts showed promising antimicrobial activity in view of bacterial pathogens. Such herbs could profit as beneficial origin of novel antimicrobial agents (10). Thus, this property can be a promising ally in the advancement of medicines necessary to combat MDR bacteria (11).

P. crispum (Mill.) commonly name as parsley. The plant came into being in the Mediterranean area of Europe and is cultivated worldwide today. It is a biennial and glabrous, has a characteristic spicy odor and grows from 60 to 100 cm high. The usually numerous stems grow from 1 root and are round, finely grooved and branched. The root is thin or thick fusiform to tuberous, vertical and almost fiberless. The leaves are ovate and tripinnate. The upper ones are shorter stemmed and less compound. The fruit is orbicularovate, 2.5 mm long and greenish-gray (12). It is commonly consumed as appetizer of the diet. In folklore

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medicines, it has been utilized as antispasmodic, uterine tonic, sedative, carminative, expectorant, antiseptic, anti-inflammatory, flatulent dyspepsia, dysuria, antirheumatic, intestinal colic, cystitis, dysmenorrhoea and myalgia (13) (14). It is a useful and significant medicinal plant with broad extent of proven pharmacological properties involving antioxidant, __hepatoprotective, neuroprotective, anti-diabetic, analgesic, spasmolytic, immunosuppressant, anti-coagulant, anti-ulcer, laxative, estrogenic, diuretic, hypotensive, gastroprotective and cytoprotective (15). The fresh or dried aerial portions of parsley considered medicinal components of the plant (12).

Inconsiderable information is defined with reference to antimicrobial impact of parsley upon medically important bacteria; hence this study is carried out with the aim of *in-Vitro* study the antibacterial action of aqueous and ethanolic extract of *P. crispum* aerial parts on certain bacteria isolated from NI.

Materials and Methods

Preparation of plant material

P. crispum was selected as a test plant. Fresh aerial parts (stems and leaves) of *P. crispum* were purchased from local market of Kirkuk, Iraq during September of 2016. Proper taxonomic identification of plant was certified by Dr. Ihsan (Education for Pure Sciences College, Tikrit University). The plant materials were washed with flowing tap water to disperse soil and other dirt, and then dried under sunlight for two days. After drying, the plant materials were crushed into delicate powder using an electrical milling machine.

Preparation of plant extracts

Aqueous and ethanol extracts of parsley aerial fractions were used for testing in the actual study. Two hundred grams of prepared dried powdered plant were weighed using the digital analytic balance (Denver instrument, Germany), then two of 100 grams portions of the dried powdered plant were soaked separately with 600 milliliter of distilled water and 99% ethanol by maceration method as described by Sing (16). The mixture was left to withstand at room temperature for 7 days with occasional shaking. The Extracted samples were filtered through

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double coatings of muslin cloth. The solvent in the extracts was completely removed prior to testing by concentrating the filtrates in oven (Memmert, Germany) at 70 °C for 48 hours in order to dry the extracted sample. The final products were stored in small airtight sterile containers at 4 °C under refrigerated conditions up to next testing.

Preparation of test sample extracts concentrations

For the preparation of concentrations of raw extracts for screening antibacterial testing, the extracts were re-dissolved into appropriate volumes of sterile distilled water as solvent and further diluted to obtain eleven different concentrations (0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 100, 150 and 200 mg/ml) of each plant extract and stored in small airtight sterile cups (size 60 ml) at 4 °C under refrigerated conditions until further investigation.

Preparation of medias

Nutrient agar (Lab M, United Kingdom), brain heart infusion broth (Rashmi, India) and mueller hinton agar (Himedia, India) were prepared in conformity with producing companies' instructions; the powder weighed, then dispersed in distilled water with aid of hot plate (IKA, Germany) and sterilized in autoclave (Trade Raypa, Spain), at 121 °C under pressure of 15 lbs for 15 minutes. Nutrient agar and brain heart infusion broth were employed for culture and diagnosis of bacteria while mueller hinton agar was used for antibiotic sensitivity testing of bacteria.

Collection of bacterial isolates

The following five multidrug-resistant bacteria were used in existing study for testing antibacterial activity of the plant extracts: *Enterococcus faecalis*, *Acinetobacter baumani*, *Morganella morgani*, *Providencia* spp and *Shigella flexneri*. All these bacteria were isolated from clinical specimens; urine (*E. faecalis*), burn wound (*A. baumani*) and stool (*M. morgani*, *Providencia* spp and *S. flexneri*) of hospitalized patients in Azadi hospital of Kirkuk.

Identification and maintenance of bacterial isolates

Conventional methods were applied for specifying the bacteria from the specimens. The plates were put in incubator (Memmert, Germany) at 37 °C for one or two days. The clinical bacterial isolates utilized in existing work were characterized pursuant to colonial, microscopic morphology and using standard biochemical tests. All positive cultures were

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activated in the brain heart infusion broth for 18 hours at 37 °C and then grown by subculturing on nutrient agar plates for overnight at 37 °C, and then the stock cultures were kept up on nutrient agar slants for every month at 4°C for subsidiary employment (17).

Evaluation of antibacterial activity

In vitro agar well diffusion method (18) according to guidelines standards recommended by Clinical and Laboratory Standards Institute (19) was assayed to determine the antibacterial activity of plant extracts opposite the experimented bacteria. For antibacterial susceptibility test, fresh culture of each bacteria that was grown for 18-24 hours was prepared, in from the four to five identical pure colonies of each isolate was transferred with sterile wire loop into tube containing 10 ml brain heart infusion broth for preparing a uniform bacterial suspension. Tubes of suspension were placed in incubator for 4-6 hours at 37 °C. Final bacterial suspensions were adjusted to reach an optical comparison to that of a 0.5 McFarland turbidity standard which is equivalent to approximately 1.5×10^8 CFU/ml (20). A disposable cotton swab was immersed into the suspension, then was streaked by threes to the surface of the plate provided with nutrient agar, the plate turned nearly 60° each time to ascertain regular dispensing of the inoculums, the edge of the agar was also swabbed at the latest step. The streaked plates were dried up in the incubator at 37 °C for 20 minutes. When the plates were solidified, a six mm diameter uniform wells were made in the surface of plates (Five wells in each plate) (21), taking on a micropipette each well was filled with 100 µl of each concentration of the herb extracts and 100 µl of distilled water representing the negative control was poured on one well. At the same time, positive controls of ciprofloxacin 10 µg disc (Bioanalyse, Turkey) and tetracycline 10 µg disc applied on the surface of plate by a sterile forceps in order to evaluate the antibacterial sensitivity of the assayed bacteria by virtue of Kirby-Bauer standardized agar disc diffusion method (22). The plates were admitted in a straight situation at 37 °C for 24 hours in incubator, and then were checked for the presence of inhibition of bacterial growth that was indicated by a visible zone encircled the wells and discs. The diameters of each inhibition zone (IZ) including the diameter of the discs and wells) on agar surface was measured in millimeters (mm) by roller and results were

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recorded. All trials were done in triplicate in the process of extreme aseptic techniques and data were analyzed.

Statistical analysis

Calculations of antibacterial activity were determined by calculating the diameter of IZ that is the mean of triplicates. The results were plotted using the Microsoft office excel computer program.

Results

Table 1 demonstrates the IZ in mm that created by each concentration of the extracts and positive controls on assayed bacteria. As noticeable in table 1, on the whole aqueous and ethanolic extracts of *P. crispum* aerial parts manifested fine antibacterial activity upon employed bacteria relating to the IZ. The aqueous extract had more effect than ethanolic extract generally speaking, particularly against *E. faecalis*, *Providencia* spp, *M. morgni* and *A. baumani*. Most likely the antibacterial influence of majority of extracts were concentration increasing dependent because the high concentration was supreme effective than the low concentration extract against each bacterium as a whole as appeared in table 1. The differences of IZ ascribed to the variations of extracts concentration for each tested bacteria. Nevertheless, rarely the increasing of IZ not has concerning with the concentration rising for example 100 and 150 mg/ml of aqueous extract against *S. flexneri* produced IZ of 19 and 18 mm alternately, like this the 8 and 6 IZ enclosing *E. faecalis* come out of 25 and 50 mg/ml of ethanolic extract.

Table 1 express that the antibacterial effect of each extract concentration varied with regard to one bacterium to another. The following concentration (0.05, 0.1, 0.5, 1 and 5 mg/ml) of aqueous and ethanolic extracts did not seem any influence upon the tested bacteria, the action began at 50 mg/ml concentration until 200 mg/ml concentration in most cases; hence the highest effect was evident upon *M. morgani* with IZ of 13, 16, 21 and 27 mm due to 50, 100, 150 and 200 mg/ml concentration of aqueous extract continually. Conversely, the 50, 100, 150 and 200 mg/ml of aqueous extract concentration manifested the lowest influence with 14, 6, 11 and 17 mm IZ confined constantly *E. faecalis*. However the 10 and 25 mg/ml of ethanolic extract was efficacious against *E. faecalis* with IZ of 5 and 8 mm respectively,

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whereas in view of *E. faecalis* and *M. morgani* the aqueous extract was effective through 25 mg/ml concentration inducing 9 and 5 mm IZ consecutively. Largely as clarified in table 1, among the checked bacteria the *S. flexneri* was the most probably affected bacteria with IZ of 17, 12, 11 and 25 mm as a result of 50, 100, 150 and 200 mg/ml of ethanolic extract consecutively, while *Providencia* spp. was the less affected bacteria according to 50, 100, 150 and 200 mg/ml of ethanolic extract that produced 5, 6, 6 and 13 mm IZ respectively. Table 1 demonstrated that antibacterial potency of 200 mg/ml ethanolic extract was identical to that of the ciprofloxacin in respect of *S. flexneri*; both of them enveloped the bacteria by 25 mm IZ. Moreover the 200 mg/ml of aqueous extract cleared up antibacterial effect on *A. baumannii* better than to that of ciprofloxacin with IZ of 21 and 15 mm one after the other.

Table 1: Antibacterial activities profile of parsley aerial parts extracts at various concentrations by agar well diffusion method and standard antibiotics by agar disc diffusion method towards five investigated bacteria.

Test extracts	Concentration	Inhibition zone diameters (mm)				
		<i>S. flexneri</i>	<i>E. faecalis</i>	<i>Providencia</i> spp.	<i>M. morgani</i>	<i>A. baumannii</i>
Aqueous	0.05 mg/ml	-	-	-	-	-
	0.1 mg/ml	-	-	-	-	-
	0.5 mg/ml	-	-	-	-	-
	1 mg/ml	-	-	-	-	-
	5 mg/ml	-	-	-	-	-
	10 mg/ml	-	-	-	-	-
	25 mg/ml	-	6	-	5	-
	50 mg/ml	10	9	7	13	9
	100 mg/ml	19	11	17	16	17
	150 mg/ml	18	14	18	21	20
200 mg/ml	20	17	24	27	21	
Ethanolic	0.05 mg/ml	-	-	-	-	-
	0.1 mg/ml	-	-	-	-	-
	0.5 mg/ml	-	-	-	-	-
	1 mg/ml	-	-	-	-	-
	5 mg/ml	-	-	-	-	-
	10 mg/ml	-	5	-	-	-
	25 mg/ml	-	8	-	-	-
	50 mg/ml	11	6	5	5	5
	100 mg/ml	12	13	5	6	12
	150 mg/ml	17	13	6	11	10

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	200 mg/ml	25	15	13	20	11
Ciprofloxacin	10 µg	25	18	30	32	15
Tetracycline	10 µg	-	8	-	9	-
Distilled water		-	-	-	-	-

*All information is offered as average values of three triplicates of inhibition zones.

* (-): No inhibition appeared.

Discussion

Understanding the enormous advantages and risks of herbal remedy requires worldwide standardization and adequate academic experiments. Consolidations of complementary and traditional medicaments should conduct simultaneously to substantiate best applications approved by scientific approaches (23). A study revealed the uncontrolled practice of herbalists in Iraq. This necessitates the stabilizing of a control system covering proper inspection and licensing of the herbalists (24). In present research, the aqueous and ethanolic extracts of aerial constituents of *P. crispum* presented obvious antibacterial influence upon assayed bacteria in the main. Several studies mentioned the identical findings through proving the effects of diverse part of parsley using various extracts against different bacteria; Al-Kareemi clarified that Parsley juice has potent effect against both gram positive and gram negative bacteria causing of urinary tract infections *in vitro*. Most bacterial isolates showed IZ to Parsley juice with different diameters (21). A research revealed that hot and cold water extracts of *P. crispum* leaves were active upon *Salmonella* spp. and *Enterobacter* spp. The potency of hot water extract was preferable on most treated bacteria compared with cold water extract (25). Distinct bioactive substances have been defined in *P. crispum* such as phenolic compounds particularly flavonoids (such as apigenin, apiin and 6"-Acetylapiin), essential oil components (mainly myristicin and apiol), coumarins and furocoumarins (15). The pharmacological features of parsley are primarily associated with the essential oil, particularly the apiole, myristicin and furanocoumarin constituents. Most of the reported uses of parsley are perhaps in consequence of the volatile oil (14). Commonly the ethanolic extract

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was less potent than aqueous extract, which is in contrast with Alshwaikh *etal.* survey when the IZ produced by ethanolic extract were broader than that come out of aqueous extract in most cases with 12.5, 25, 50, 75 and 100 mg/ml concentration of each them expressing the priority of ethanolic extract against experimented bacteria (26), but the result is in compatible with findings obtained by previous study relying on applying aqueous and ethanolic extracts of parsley with 2.5, 5, 10, 20 and 40 mg/ml concentrations upon numerous bacteria. The effect of aqueous extract was better than ethanolic extract as a whole, attributed the reasons to the quality of ethanolic extract that spread slowly in the agar or owing to tender herbs comprised efficient substances which may be affected or disappeared by the steps of extraction methods (27). The strength of antibacterial activity was in accordance with extracts concentration excess at large, as also recorded by works of (28-30); Ashour and Astal stated that 125, 250 and 500 mg/ml of parsley aqueous leaf extract possessed antibacterial effect with 9, 12 and 14 mm IZ respectively toward *Pseudomonas aeruginosa*, whereas extract of ethanol 96% produced IZ of 8, 10 and 13 mm due to antibacterial influence facing *Enterococcus* sp. out of using 12.5, 25 and 50 mg/ml concentration continually (28). The methanol extracts of *P. crispum* leaves exhibited antibacterial activity facing tested bacteria, three concentrations (100, 250 and 500 µg/disc) of the extract provoked IZ as following: 8.5, 10.3 and 13.4 mm consecutively on *P. aerogenosa* and 11.4, 13.6 and 16.7 mm one by one around colonies of *Staphylococcus aureus* (29). In another research work, it was observed that 75% concentration of parsley aqueous extract induce 9 mm IZ about *Escherichia coli* colonies, IZ of 10 mm against *E. coli* and *Klebsiella pneumonia* originated by 100% concentration (30). Though the intensity of antibacterial effect not with regard to increasing of the concentration in limited cases in current study, the investigations of Seyyednejad *etal.* and Al-Hadi *etal.* support this result; the ethanolic extract of *P. crispum* seed inhibited the growth of diverse species of gram positive and gram negative bacteria. IZ created toward *E. coli* were 12, 10, 10 and 9 mm on account of 100, 200, 300 and 400 mg/ml concentration by turns, whilst *Brucella melitensis* affected by 100 and 200 mg/ml concentration causing 9 and 7 mm IZ one after the other (31), the growth inhibition of examined bacteria was very strong in consequence of hydroalcoholic extract of parsley leaf, IZ surrounded colonies of *E. coli* were

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17, 10 and 12 mm using 250, 500 and 1000 µg/ml concentration constantly (32). Very frequently the aqueous and ethanolic extracts did not exhibit any action on the used bacteria with the following concentration (0.05, 0.1, 0.5, 1 and 5 mg/ml), the effects started at 50 mg/ml concentration till 200 mg/ml concentration. This result is in agreement with report of Ljubiša *etal.* when they approved that ethanolic extract of parsley fruit did not show antibacterial activity at lower concentrations (50, 100 and 250 mg/ml) continually, while 500 mg/ml concentration exhibited intermediate and moderate antibacterial effects upon investigated bacteria (33), study of Petrolini *etal.* also confirmed this result as the crude ethanolic extract derived from the leaves and stems of *P. crispum* furnished minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) higher than 0.4 mg/ml for entire explored bacterial species, except for *P. aerogenosa*, which created MIC and MBC of 0.35 mg/ml and above 0.4 mg/ml, respectively, that resulted in a bacteriostatic effect (34). On the other hand, recently a paper mentioned that 0.2% yielded parsley essential oil has antibacterial activity; the growth of all tested bacteria were inhibited with MICs ranging from 0.04 to 1.00 mg/ml and killed with MBCs ranging from 0.15 to 10.00 mg/ml (35). Hence; it is probable that the minimal antibacterial effect recognized in current study may be due to apply of whole plant extract, which could have lower concentration of bioactive components when compared to essential oil. Clearly displayed the antibacterial action of higher concentration (200 mg/ml) of the extracts was optimum or parallel to that of ciprofloxacin against specific same bacteria, the same notice was reported by Alshwaikh *etal.* when they observed that the antibacterial action of the final concentration (100 mg/ml) of ethanolic extract was equal to that of impinem as positive control face to *Proteus vulgaris* with IZ of 22 mm, as well as the influence of the similar extract with 75 and 100 mg/ml concentration toward *P. mirabilis* were superior to that of impinem with 18, 20 and 15 mm one after the other (26). Occasionally, the herb extracts that comprised the phytochemicals had higher IZ than an antibiotic, meaning propitious signs for antibacterial activities of such extracts (9).

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-Conclusions

Results of current study concluded that aerial portions of *P. crispum* have excellent antibacterial effect that affirms their consumption in foodstuff, clears away for their applying as antibacterial agent especially against NI. In conformity with previous informations, additional researches are required to segregate the phytoconstituents from *P. crispum* aerial parts then evaluate their activity towards MDR bacteria that relate to NI.

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