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Effect of Four Plant Extracts on Opportunistic Bacteria: *Sphingomonas paucimobilis* and *Enterococcus faecium*

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Abstract

The emergence of new bacterial species in different infections, such as *Sphingomonas paucimobilis* and *Enterococcus faecium* needs materials to get rid of, especially if these materials are of 100% natural origin, however, repeated experiments must be conducted from different materials for the same bacterial species until reaching the target material. The research has investigated about if two nosocomial infective bacteria *Sphingomonas paucimobilis* and *Enterococcus faecium* could influence with some plants (*Lepidium sativum, Sinapis arvensis, Eruca sativa and Raphanus sativus*) belong to the Brassicaceae family which are mainly known containing compounds that are effective in combating pathogenic bacteria, using the disk diffusion method. The result was no inhibition zone around the disks saturated with water and alcoholic extracts separately by all plants against these two bacterial species tested. The authors concluded that these bacteria might have adaptation from previous exposure to their environment. From our result, it is clear that there is a need to test extracts from other plants to resist these two bacterial species, which may pose a health risk, especially since they are previously registered to be resistant to antibiotics.

Keywords: *Sphingomonas paucimobilis, Enterococcus faecium, Lepidium sativum, Sinapis arvensis, Eruca sativa and Raphanus sativus.*

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I. INTRODUCTION

Sphingomonas paucimobilis (*S. paucimobilis*) are non-fermented Gram-negative rods recognized as rare causes of predominantly nosocomial infections (Ionescu *et al*., 2022), the understanding of the epidemiology of *Sphingomonas* species infections is based on case reports and small hospital-based series at high risk for selection biases (Al-Hasan *et al*., 2011).

There is a chance that the epidemiology in any given area will be unique from that in other areas (Laupland *et al*., 2022). The bacterium may adapt to a wide range of variable conditions including: temperature, carbon sources, pollutants, etc (Jardine *et al*., 2017). Enterococci are members of the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, Enterobacter spp.) which have been highlighted by the WHO as a growing threat to public health in recent years due to the development of nosocomial and antibiotic-resistant infections (Zhen *et al*., 2019). Commonly, Enterococci are considered as opportunistic infections for humans worldwide and are in charge of bacteremia, infections of the urinary system, the heart, and very infrequently meningitis and intra-abdominal infections (Arias and Murray, 2012).

Brassicaceae (Cruciferae) family is one of the economically and ornamental important plants, known as mustard family, includes 338 genera and 3709 species. The presence of the compound glucosinolate distinguishes plants in the Brassicaceae family is concentrated in the seed. Isothiocyanates (ITCs) compounds derived from glucosinolate have been shown in both *in vitro* and *in vivo* investigations to have positive effects on human health, including anticancer,

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antibacterial, and antiviral activity (Quirante-Moya *et al*., 2020; Sikorska-Zimny and Beneduce 2021).

The antimicrobial behavior of these compounds from plants in the Brassicaceae family against bacteria received little attention although ITC demonstrated its biocidal activity against *Staphylococcus aureus* and *Escherichia coli*, *Helicobacter pylori* as well as *Campylobacter jejuni* (Ugolini *et al*., 2021).

Due to the mentioned of the infections caused by *S. paucimobilis* and *Enterococcus Faecium* (*E. Faecium*) which have recently started to increase to humans, and due to the fact that the plants of the Brassicaceae family contain deadly compounds for bacteria, so the authors have tried four different species of them (*Lepidium sativum*, *Sinapis arvensis, Eruca sativa and Raphanus sativus*) in their ability to inhibit the growth of bacteria *in vitro*, in an attempt to limit the spread of infections caused by these bacteria in a safe and common way.

II. MATERIALS AND METHODS

A. Bacterial isolates

Two bacterial species: *S. paucimobilis* and *E. faecium* were obtained from (department of Biology/College of science/University of Mosul) and isolated from pathogenic cases (Otitis media), Although the biochemical tests were done to ensure the identity of isolates according to (de la Maza *et al*., 2020) like catalase, oxidase, urease, novobiocin and bacitracin sensitivity and citrate utilization tests, then these two bacteria were sent to a private laboratory to diagnose them by vitek-2 apparatus.

B. Plants

four plants from Brassicaceae family were used in this study:

- *Lepidium sativum* (water cress)
- *Sinapis arvensis* (mustard)
- *Eruca sativa* (water cress)
- *Raphanus sativus* (Radish)

Seeds samples were bought from local markets in Mosul, Iraq, grinded in grain grinder type (powder grinder copper motor) until it became powder.

C. Extracts preparation

For the preparation of both ethanolic and aqueous extracts of four plants, 10 g of plant powder was soaked in 100 ml of each solvent (ethanol and sterile distilled water) separately for 3 days with sterior and then filtered through an eight-layer muslin cloth. They were further filtered with filter paper (Whatman No. 1) and centrifuged at 3000 rpm for 10 minutes. The supernatants were collected separately and stored in sterile bottles at 4 °C. Then the extracts were poured into glass dishes and left to dry. After the extract was completely dried, 10 ml of distilled water was placed over the plant extract, along with 10 ml of Dimethyl sulfoxide (DMSO) on top of the alcoholic extract. Then each extract was put into a sterile tube (Akrayi and Tawfeeq, 2012).

C. 1 Sterilization and Preparation of extracts disks

Each of the aqueous and alcoholic extracts of the plants used was sterilized by Millipore filter paper size 0.45 mm in diameter. To prepare the extract disks, filter papers have been cut manually to form discs with 6 mm diameter and sterilized using an autoclave, then groups of discs were distributed in sterile dishes and each group was soaked with a particular extract separately and for drying placed in the incubator for a period 24 hours.

D. Disk diffusion method

This experiment, depending on Kirby and Bauer (1966) with some modifications, was achieved to detect the ability of prepared plant extracts to inhibit the growth of bacteria under study as follows:

- Each bacterium was grown in a nutrient broth medium for 24 hours at 37° C.
- 100 µl of bacterial suspension (1.5×10^8 cell /CFU) compared with MacFarland tube No. 0.5 was spread by L-shaped on Muller Hinton Agar.
- By using sterile forceps, the disks previously prepared from plant extracts were set on culture, then incubated for 24 hours at 37 ° C.

Al-Mansur Laboratory

bioMérieux Customer: **Microbiology Chart Report**

Patient Name: 1, 1 — Patient ID: 427
Location: — Physician:
Lab ID: 427 — Isolate Number: 2

Organism Quantity:
Selected Organism : Sphingomonas paucimobilis

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:** 5.78 hours	**Status:** Final
Selected Organism	97% Probability **Sphingomonas paucimobilis** **Bionumber:** 5000001000200001	
ID Analysis Messages		

Biochemical Details																													
2	APPA	+	3	ADO	-	4	PyrA	+	5	IARL	-	7	dCEL	-	9	BGAL	-												
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	-	14	GGT	-	15	OFF	-												
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-												
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	-												
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-												
40	ILATk	-	41	AGLU	+	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-												
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-												
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-															

Figure 1: The report of Vitek-2 system microbiology chart for *Sphingomonas paucimobilis*

III. RESULTS

A. Identification of bacterial isolates

In the present study, all isolated bacteria were identified up to the species level including

S. paucimobilis and *E. faecium* using automated Vitek-2 systems as demonstrated in Figure 1 and 2 respectively after the diagnosis of bacteria by using biochemical reactions.

B. Disk diffusion method

This method was used to reveal the influence of the aqueous and ethanolic extracts of four plants from the Brassicaceae family:

- *Lepidium sativum*
- *Sinapis arvensis*
- *Eruca sativa*
- *Raphanus sativus*

against two pathogenic bacteria *S. paucimobilis* and *E. faecium* with a concentration 1.5 x 10^8 cfu/ml, the results unfortunately showed that there was no inhibitory effect of both aqueous and alcoholic extracts for all four plants against these two bacteria.

Al-Mansur Laboratory

bioMérieux Customer: Microbiology Chart Report

Patient Name: 2, 2 Patient ID: 428
Location: Physician:
Lab ID: 428 Isolate Number: 3

Organism Quantity:
Selected Organism : Enterococcus faecium

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	4.80 hours	**Status:** Final
Selected Organism	98% Probability **Bionumber:**	**Enterococcus faecium** 130002045373671	
ID Analysis Messages			

Biochemical Details

2	AMY	(+)	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	+	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	+
38	dRIB	+	39	ILATk	-	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	-
47	NOVO	+	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

Figure 2: The report of Vitek-2 system microbiology chart for *Enterococcus faecium*.

IV. DISCUSSION

In the present study, the diagnosis of *S. paucimobilis* and *E. faecium* - isolated from otitis media - have been confirmed up to species level using automated Vitek-2 systems.

Species identification of these bacteria can be challenging due to their phenotypic and biochemical similarities with other species. The use of conventional biochemical tests for diagnosis may have limitations and could result in misidentification. It is important to consider the potential drawbacks and limitations of traditional identification methods especially in a clinical or research setting where accuracy is crucial. For this, the vitek-2 apparatus was used because without further information by this system, the identification cannot be definitively confirmed, although the vitek-2 systems is used to identify only a few of the Enterococcus species.

Up until 1977, the Gram-negative rod *S. paucimobilis* was classified as a member of the genus Pseudomonas; it is strictly aerobic and non-fermentative. *S. paucimobilis* strain Kira's whole genome sequence has just been published, the strain has 3,917,410 base pairs and a G + C composition of 65.7% (Nishimura *et al*., 2021). The lipopolysaccharide of *S. paucimobilis* may have undergone certain structural changes as a result of the organism's adaptation to its surroundings. Infections caused by *Sphingomonas* species are typically recognized as nosocomial (hospital-onset) pathogens (Kawahara *et al*., 1999).

Enterococci are considered as a useful sign of environmental fecal contamination because of their presence in animal stool and environmental tenacity, enterococci can be spread in many of locations, and they can be isolated from water, plants, food of animal origin, soil, and sewage (Byappanahalli *et al*., 2012).

Both nosocomial infections and community-acquired illnesses are caused by enterococci, an opportunistic pathogen. Two species, *E. faecalis* and *E. faecium*, are regarded as the most significant nosocomial pathogens globally (Guzman Prieto *et al*., 2016), however, there has been an increase in reports of non-faecium and non-faecalis enterococci infecting people's blood, urinary tracts, and surgical wounds (Monticelli *et al*., 2018).

We interpret our results as meaning that the bacteria isolated from local cases have developed adaptation to what is present in their local environment, this explanation agrees with Laupland and his colleagues (2022) who indicated that it is possible for the bacterial epidemiology in one area to be different from that in another.

Our findings did not agree with previous researches, which mentioned that these plants are considered medical plants because of their ability to face many pathogenic bacteria as well mentioned below:

Khatib and Al-Makky estimated the content of phenols and flavonoids of the parts of the mustard plant *Sinapis alba* separately and measured the inhibitory effects of their extracts on bacteria and found that phenolic extracts are the best effective in preventing bacterial growth, as the inhibition's diameter area was 17.2 mm for leaf extracts, as the inhibition area's diameter reached 15 mm for flower extracts, and it was found that the highest phenolic flavonoid content is present in dried flower extracts (Khatib and Al-Makky, 2021).

In a study by (Besufekad *et al*., 2018) on the effect of methanolic cress seed extracts, ethanolic and chloroform on pathogenic *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *S. aureus* and *Shigella sonnie*. Using the well diffusion method, ethanolic and methanolic extracts showed the highest effect on *E. coli* bacteria with an inhibition area equal to 22 mm, while methanolic extracts of cress showed the least effect on *Pseudomonas aeruginosa* bacteria, the inhibition area was 9 mm and the researchers hypothesized that the cress extracts in ethanol and methanol could be utilized to treat infections caused by *E. coli* and *Pseudomonas aeruginosa*.

Lipidium sativum shows activity against pathogenic bacteria *S. aureus*, *S. epidermedis*, *S. saprophyticus*, *Klebsilla*, *Provedenatia*, *Pseudomonas*, *E. coli*, *Proteus*, *Serratia* because it contains phenols, alkaloids and terpenoids, where it showed that all bacterial species are resistant to phenolic compounds, while the genera *S. aureus*, *Klebsilla* and *Provedenatia* showed resistance to all compounds in cress extract while Alkaloids and terpenoids extracted from cress showed a wide range of antibacterial efficacy.

Lepidium sativum plants and seeds are among the common natural remedies that are effective in treating bone fracture healing. A study by Adam *et al*., (2011) mentioned the traditional applications of the seed extract of *Lepidium sativum* in solving medical issues. They were employed as antiscorbutics, diuretics, aperients, poultices, and stimulants. The leaves have stimulant, diuretic, and antiscorbutic properties, it was established that the aqueous extract of *Lepidium sativum* significantly lowered blood pressure.

Ahmad *et al*. (2012) mentioned that *Raphanus sativus* (Radish) is useful for piles and urinary complaints; all plant parts are used in medicine. The fresh juices from the leaves are used as a diuretic, laxative. Roots are useful for syphilitic disease and urinary complaints, as well as medicine for gastronomic pains and piles. The seeds are diuretic, expectorant laxative, antitussive, carminative, and stomach tonic.

Akrayi and Tawfeeq detected the antibacterial effects of aqueous and ethanolic (cress garden) extracts of *Lepidium sativum* and (leek) *Allium porrum*, and their juices, on Gram positive and negative bacteria (*Staphylococcus aureus*, *Proteus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*), they found that all of the bacteria under research, with the exception of *Klebsiella pneumoniae*, were inhibited by plant extracts, whereas the juices of both plants had no impact on the bacteria (Akrayi and Tawfeeq, 2012).

Eruca sativa seed extracts and seed oil were examined for their antibacterial activity against Gram positive and Gram negative bacteria by (Gulfrazi *et al*., 2011). In comparison to broad spectrum antibiotics like gentamicin, seed oil showed the greatest suppression of all bacterial strains, followed by methanolic seed extracts, other essential and non-essential fatty acids was also present in trace amounts, the research supports up the efficacy of *E. sativa* seeds as a traditional treatment for a variety of disorders.

The charlock mustard *Sinapis arvensis*, wild mustard, is a winter annual plant, that have an erect stem with a coarse hairs and rasceme inflorescence composed of yellow flowers (Parnell and Curtis, 2012; Al-Qudah *et al*., 2011) analyzed the chemical composition with GC \ MS and antimicrobial activity of essential oils from the shoots of *S. arvensis L*. and *S. alba* L. and, they revealed that the mixture was composed of nitriles, aldehyde, sulfur compounds, mono - and sesquiterpenes, and many other compounds, essential oils antibacterial activity of the two species showed that *Staphylococcus epidermidis* is the most susceptible of Gram negative and Gram positive bacteria.

In a study of the impact of mustard plant extracts on three bacterial strains, the oil of mustard had an antibacterial effect because it contained allylisothiocyanate. It was found that all plant parts of the *Sinapis alba* plant have an antibacterial effect against *S. aureus* but do not exhibit an inhibitory effect on either *E. coli* or Pseudomonas (Camacho *et al*., 2019).

In a study (Ashebir and Ashenafi, 1999) on the effect of some medicinal plants, including the aqueous extract of cress on foodborne microbes, including *Bacillus cereus*, *S. aureus*, *Shigella boydii*, *Shigella flexineri*, *Salmonella typhimurium*, and *Escherichia coli*, they stated that aqueous alveolates can cause delayed bacterial growth rather than inhibit them.

The antibacterial activity of ITC (present in a plant of the Brassicaceae family) appears to have a multitargeted mechanism of action in various bacterial strains, affecting a number of metabolic pathways, and most likely causing membrane damage, inhibiting enzyme activities like cellular respiration, inducing of heat-shock and oxidative stress responses, and depleting free amino acids (Nowicki *et al.*, 2021).

From our point of view as academic researchers, until effective compounds which *S. paucimobilis* and *E. faecium* be sensitive to them will be discovered, it may be necessary to use plants from other families (Ahmad and Mohammad, 2019) or to search for active ingredients alone, or combine them with other materials like nanoparticles (Selah and Mohammad, 2021), in order to use them as a promising treatment for a variety of infections caused primarily by bacteria and avoid developing pathological issues.

V. CONCLUSION

The lack of lethal effect by various extracts from different plants of the Brassicaceae family (which is well known in their activity against varying microorganisms) on *S. paucimobilis* and *E. faecium* is considered dangerous result.

From our findings, the concerns about the absence of impact the plant sources on pathogens may start, because the plants remain the basic and essential source for fighting the etiological agents, especially after the outbreak of antibiotic resistance and the emergence of Multidrug-Resistant and extensively drug-resistant strains, where natural plants and herbs are used as alternatives to antibiotics, and here we note the great danger of resistance of *S. paucimobilis* and *E. faecium* even against the natural compounds found in these

four plants. Therefore, we recommend using plants from other hosts until effective compounds are found that are bacteria sensitive to them, investigating active substances alone or mixing them with other materials such as nanoparticles in order to be used as a promising treatment in various infections caused mainly by bacteria and avoid falling into pathological problems.

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