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Antibacterial Compound of Mixture Turnip and Honey for Some Microorganisms

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Abstract:

Objective: investigate the ability of turnip and Honeyextracts against numbers of microorganisms (Salmonella Enteritica, Streptococcus penemuim).
Methods: In this research, the impact of different concentration of mixture of turnip and honeyextracts on Streptococcus pneumonia (5, 10 and 15$\mu L, mm$) ,Salmonella enteritica (15, 20 and 25 $\mu L, mm$) were examined. The antibacterial activity was studied using disk diffusion. Thedata analysed by one-way *ANOVA* and Probit Analysis via the SAS software.
Results: The diameter of zone of the inhibition of honey and turnipextractshas tested,obtained ranged 36-46 mm for Salmonella Enteritica, 36-56 mm for Streptococcus penemuim.
Conclusions: A profound synergistic effect of honey and turnip was observed and the data obtained in vitro showed that turnip plant and honey have good antimicrobial potential and can be used because it is natural in combination with other foods.

Key words: Honey, Turnip, Antibacterial activity, salmonella, mixture of turnip and Honey.

Introduction:
Here are more than 400,000 plant species worldwide that have antibacterial properties and activities but have not been studied yet (sharma, 2009).plants have the potential to produce Secondary metabolisms andcomplex compounds that have antibacterial properties (shu, Alizade, callnic).Plants of the genus *Brassica* belong to the family *Brassicaceae* and include several of the most commonly consumed vegetables all over the world. Some researchers claim that turnips have been eaten since the 1500s, while others believe the plant to date back to prehistoric times (Polidoro, 2008). Brassica species include: *Brassica juncea*, *Brassica napus*, *Brassica rapa*, *Brassica oleracea*. *Brassica Rapa* (*turnip*) has been cultivated and consumed for many centuries across Europe and expanding eventually to central and East of Asia (Hassanzadeh-Taheri et al 2018) and include several of the most commonly consumed vegetables all over the world. Among them *'turnip' Brassica rapa* L, which is highly consumed as edible vegetable ((Beltagy, 2014). Turnip edible parts are consumed as a raw, boiled and/or fermented vegetable all over the world. They contain a variety of organic compounds with biological activity such as *glucosinolates*, *phenylpropanoids*, *flavonoids*, *phenolics* and organic acids .Also, the *ethanolic* extract of turnip roots exerted anti diabetic activity in all oxidized diabetic rats. Many of the proven biological activities of turnip can be related to antioxidant potential. Other research revealed that the ethanol extract of the roots possessed antioxidant potentials such as free radical scavenging, nitrite scavenging, and lipid peroxidation inhibitory activities as well as reducing power and lipid peroxidation inhibition (Beltagy, 2014 and Dastan et al 2006 and Ahmadvand et al 2008).The medicinal properties mentioned for the turnip have prompted the researchers to investigate its antimicrobial activity. Methanol, ethanol, n-hexane and chloroform extracts of turnip were effective against Pseudomonas aerogenase (Dasatan et al, 2011), Beltagy

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showed antimicrobial effect of cured turnip extract on Pseudomonas aeruginosa (Beltagy, 2014).
Natural compounds such as honey obtained from plants have a direct positive effect on human health (Khansaritoreh et al, 2021).Honey issupersaturated sugar solution, which is produced by honeybees, is the main concentrates found in carbohydrate honey including glucose, fructose and sucrose.In addition, honey contains a variety of minor compounds such as pigments (carotenoids and anthocyanins), phenolic compounds (phenolic acid, flavonoids), amino acids, vitamins (C and E), enzymes, organic acids and minerals (Almasudi, 2021; Saxena et al, 2010; Djebli et al, 2020).Honeyhave anti-inflammatory properties (Tonks et al, 2003), healing (Bergman et al, 1983), antioxidant (Jaganathan et al, 2010) and antineoplastic (Swellan et al, 2003).Since previous times honey has been used to treat bacterial infections, colds and coughs, a variety of infectious diseases.Production of hydrogen peroxide (H2 O2), bee defensive, high osmosis and low pH are factors that affect the antibacterial properties of honey.Other phytochemical factors, especially phenolic compounds, tetracycline, peroxidases, amylases, fatty acids, ascorbic acid, terpenes, benzyl alcohols and benzoic acid make honey a substance that has antibacterial ability against gram-positive and gram-negative pathogenic bacteria (Almasudi, 2021).Extensive research has been conducted around the world on the antibacterial effect of honey and has proved this property.Escherichia Coli and Pseudomonas Aeruginosa and Salmonella Entrica (Mandal et al., 2010), Staphylococcus aureus and Streptococcus piogenosa (Moussa,et al., 2012), Streptococcus mutans (Rezvani et al., 2017)
Salmonella enterica and Streptococcus pneumoniae are known as two pathogens.Considering the importance of finding natural ingredients to fight pathogens, it seems that the use of two natural substances, turnips and honey, which have been used as natural medicines from a distant past, seems to encourage the use of two natural substances, turnips and honey.The aim of this study was to investigate the simultaneous antimicrobial effect of honey and turnip extract to use this property in the right way to against with pathogens.

Materials and methods:

Preparation of honey:

Honey used in this researchfrom the mountainous regions of Iraqi Kurdistanthat preparationorganically by honeybees from the natural flowers of these regionsto be prepared.

Preparation of turnip:

Turnips used in testing were purchased from local markets

Preparing of Turnip and Honey extracts:

To prepare the extract of turnips and honey, it was done by spooning the inside of the turnipwas drained almost to the middle of the turnipand a spoon of honey was poured into the cavity and allowed to remain in this state for 15 hours.After the mentioned time, the extract was emptied and it was used to evaluate the antibacterial activity.

Preparation of tested microorganisms:

Two species of microorganisms tested in this study were obtained from the Iranian mold and Bacteria Collection Centre of Scientific and Industrial Research Organization, which included:

- Streptococcus pneumonia ATCC 49616
- Salmonella entritica subs entritica ATCC 14028

Preparation of Mueller Hinton agar (MHA):

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Halt 38 milligram of the medium in one litre of clean and filtered water, put it on the cooker with frequent agitation, and boil for one minute to completely melt the medium. Autoclave at 121c For 15 minutes, then pour cooled Mueller Hinton agar into sterile Petri dishes.

Direction:

Suspend 40gram dried-up media in 1 litter of sterilized filtered water, sterilize at 121c for 15 minutes. Wait until cool to 45-50c, then mix gently and dispense into sterile Petri dishes sterile culture tubes.

Antibacterial activity test:

During the preparation of soybean casein peptone agar adding to petri dish after cooling adding the bacteria Streptococcus pneumonia spray by (L) shape and split petri dish into 4 parts and place it into 4 isolated blank parts and add the mixture of the invention that has been transferred to the disc at a concentration of (5,10,15) ml.

For the salmonella enterica:

During the preparation of muller Hinton agar Add to inside the plates after cooling adding the bacteria spray by (L) shape and divide petri dish, in 4 parts and place in each part a disc blank and add the mixture to the discs at concentration (15,20,25) ml.

Statistical analysis:

All tests were performed in Triplicate. Results were analyzed by one-way ANOVA and Probit Analysis via the SAS 9.2 software.

Figure 1- Preparing of Turnip and Honey extracts

Results:

Sensitivity of Salmonella enterica and streptococcus penemuim to concentrations (5, 10 and 15 for streptococcus penemuim) (15, 20 and 25 for Salmonella enterica) of turnip and honey extract were studied.Tables 1 and 2 show inhibitory diameters on these two gram-positive and gram-negative bacteria.As it is understood from the tables, the effect is increased by increasing the concentration of the extract.Streptococcus penemuim showed lower amounts of sensitive to extract compared to Salmonella entretica.Diameter of the inhibition zone for streptococcus penemuim was 20-56 mm and

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Salmonella was 36-46 mm.

Treatment of streptococcus penemuim			
Antimicrobial agent	Treatment Concentration $\mu L, mm$	Mean±standartdivition(mm)	Tukey grouping
Honey and Turnip extract	5	20.23±0.24	Cd
	10	44.23±0.23	C
	15	56.23±0.24	B

Table 1:Mean diameter of growth inhibition zone obtained from different concentrations of honey and turnip extract for streptococcus penemuim

Treatment of Salmonella enterica			
Antimicrobial agent	Treatment Concentration $\mu L, mm$	Mean±standartdivition(mm)	Tukey Grouping
Honey and Turnip extract	15	36.65±1.25	¥gh
	20	41.67±0.01	De
	25	46.00±0.05	C

Table 2: Mean diameter of growth inhibition zone obtained from different concentrations of honey and turnip extract for salmonella entertica

Discussion and conclusion:

It can be claimed that the extracts of plants and natural substances such as honey and turnip have been used by human for thousands of years due to their medicinal and food properties.There are many reports and research to prove the antimicrobial and antibacterial effects of extracts obtained from natural ingredients (Prabuseenivasan et al, 2006).

According to our results, turnip and honey extracts have the ability to inhibit the growth of Salmonella enterica as a gram negative bacteria responsible for salmonellosis, which is the most importance zoonotic disease in public health, responsible for outbreaks of gastrointestinal illness in humans and Streptococcus pneumonia as a Gram-positive bacterium is an important pathogen that causes serious invasive infections, such as *septicaemia*, *meningitis* and *pneumonia*, and mild upper respiratory infections., which explained the reasons as follows. These antibacterial effects of plants are due to the presence of chemical compounds or secondary metabolites such as alkaloids, flavonoids, glucosides, terpenoids, polyphenols and steroids. Turnip plant has phenolic and flavonoids compounds and this plant converts it as a substance with antioxidant and antibacterial properties.Honey also offers antibacterial potential due to the ability to produce hydrogen peroxide and create high osmosis and low pH and the presence of phenolic compounds.

The reason why different bacteria have different reactions to the extracts can be noted that they have a different structure in terms of wall structure. The presence of liposaccharide walls in gram-negative bacteria acts as a barrier to the passage of molecular substances of large and hydrophobic substances, and since most plant extracts have hydrophobic properties, they are not allowed to pass through the bacterial cell and disrupt the function of gram-negative bacteria. As can be seen in this study, Salmonella enterica shows higher resistance to the extract compared to Streptococcus pneumoniae(Yahya-abadi et al, 2011).

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Research conducted over the years in different parts of the world has results in order to confirm the resultsof this research. In 2017, Behnam et al. investigated the chemical composition and antimicrobial properties of essential oil of Shirazi turnip root their results showed that the essential oil of Shirazi turnip had a significant effect on the reduction of E. coli, Staphylococcus aureus bacteria (Behnam et al, 2017).A study conducted by Beltagy in 2014 to investigate the antimicrobial and antioxidant properties of turnip root and shoot extracts with various solvents .The results showed inhibition of turnip extracts with different solvents on Candida albicans, Pseudomonas aeruginosa and Bacillus cereus.In 2010, Hassanian et al. reported the effect of honey on Streptococcus aureus in vitro.

Finally, these results indicate that turnip plant was used as an antimicrobial agent and due to the antimicrobial effects of turnip, identification and evaluation of the effective compounds of these plants is essential. Considering the fact that the amount of compounds in medicinal plants is different according to different climatic conditions and in different parts of the plant, it is necessary to check the amount of compounds in different regions during other experiments.

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