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Effectiveness of Mixed Gycyrrhiza Glabra and Punica Granatum Plant Extract and Honey against Helicobacter Pylori Bacteria

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Abstract: Objectives: The current study intends to determine the effectiveness of honey and the plants alcoholic extracted Gycyrrhiza glabra, Punica granatum, and honey alone or in combination to determine the antibacterial action of these plants against Helicobacter pylori. Methods: For made the cured extraction of plants G. glabra and P. granatum, ethanol is the solvent used. The most effective concentration of solvent optimization was found by extracting and diluting honey, testing its efficiency against the H. pylori bacteria, and determining the "minimum inhibitory concentration" (MIC) of the additional effective extract.
Results: The extraction in vitro demonstrated effect against H. pylori in contrast to the antibiotic. Results observed in pathogenic bacteria by inhibition zone diameter the mixed plant extract of G. glabra and P. granatum with honey in concentrations (50, 25, 12.5, mg/mL) (26.20, 14.86, 8.13 mm) in arrangement, and all of this means that there is a significant variation in the P-value and little effectiveness when treated with plant extract of G. glabra and P. granatum (50, 25, mg/mL) (12.10, 5.46 mm) and with effectiveness in the mixed plant extract of G. glabra and L. usitatissimium in (50 mg/mL) (7.33 mm) without any significant variation in P-value and no effectiveness in the plant extract of G. glabra alone. Conclusion: Current studies will aid in the isolation of novel goods and medications. The findings of this study have demonstrated the presence of antibacterial and antioxidant components in three plant extracts and honey.

Keywords: Gycyrrhiza Glabra, Honey, Helicobacter Pylori, and P. Granatum.

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1. INTRODUCTION

Helicobacter pylori, also known as H. pylori, is an IARC because it causes malignant transformation when combined with common bacterial pathogens that cause multiple atrophy and persistent mucosal inflammation (1). H. pylori infection has been connected to multiple cases of gastritis and peptic ulcer disease in virulence factors and has been shown to be associated with addition (2). Moreover, the ischemic cardiovascular and cerebrovascular International Agency for Research on Cancer disorders classifies H. pylori non-gastric diseases such type 2 diabetes as class I carcinogens (3).
Medicines made from plants have been utilized for thousands of years. Since ancient times, nature has provided medical substances, and a remarkable number of contemporary medications have been derived from natural sources (4). Even now, pharmacologically active chemicals from therapeutic plants are being isolated and their biochemical characteristics are being studied (5).

2. LITERATURE REVIEW

Since at least 500 BC, licorice has been used medicinally to refer to the roots and stolons of some Glycyrrhiza species, including Glycyrrhiza uralensis Fisch. (Fabaceae) (6). an extract from G. uralensis is thought to be useful in the management of peptic ulcer illness. It has recently been discovered that this plant has biological properties that include anti-inflammatory, antioxidant (immunomodulatory), and other properties (7). Chemical components of the various Glycyrrhiza species, as well as methanol extract and flavonoids from G. uralensis, were investigated for their anti-H. Pylori properties. G. uralensis semi-purified fractions showed varying anti-H. Pylori activity. G. uralensis flavonoids were found to be efficacious against H. pylori stains in the majority of cases (8).

Chronic gastritis has been treated with Punica granatum L. (Lythraceae) in traditional Iranian medicine (9). Pomegranate peel has been shown to have antimicrobial, antiparasitic, antiviral, antioxidant, and anti-inflammatory properties. Additionally, by making H. pylori strains more hydrophobic on their cell surfaces and preventing the bacterium from adhering to the stomach mucosa, the plant can reduce the issue of H. pylori drug resistance (10).

In recent years, honey has gained more and more attention as a "natural" remedy for bacterial illnesses. Honey has demonstrated efficacy against a variety of bacteria, and reports of its ability to inhibit the growth of particular microbes have received widespread validation (11). The beneficial effects of honey on the wound environment are widely recognized. Honey's extreme stickiness helped to provide a protective barrier to avoid infection and controlled the wet wound environment that aided in healing. Many people in Cameroon frequently use it for its antibacterial qualities because it is easily available and affordable. Honey is used to treat gastrointestinal issues and as an antibacterial for burns and wounds (12, 13)...

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3. MATERIALS AND METHODS

Plants Material

The Kerbala government regional market gathered honey, P. granatum Peel, and dried plant pieces of G. glabra. Plant parts were then dried for weeks at 45ºC in an oven before being transported and crushed, either manually or mechanically, to produce powder.

Bacterial Isolation

The gastrointestinal tract center (G.I.T.) at Al-Hussany General Hospital in Karbala, Iraq, collected biopsy samples from adult male and female subjects, aged 10 to 80, who were included in the study of dyspepsia patients' specimens. The tubes grow culture in selective media for H. pylori, after an incubated period Columbia agar with horse lysis blood, using biochemical methods to confirmatively identify H. pylori bacteria. A biopsy toke from each patient, is collected to bacteria cultured, in the brain heart in fusion media.

Culture Media Prepared

Brain Heart Infusion Media

The broth was made in one liter of distilled water by dissolved 37 grams of powder, heating the mixture until it dissolved, and then adding a supplement of H. pylori before sterilizing. As per Oxiod business protocol, isolates were preserved using this medium.

Agar Columbia

In accordance with Oxiod Company protocol for isolated pure bacteria colony, agar made through dissolved 39 g of media in 1 liter of distilled water, media boiling for dissolved, adding supplement for H. pylori then steriliz, and after cooling added 20–30 mL of lysis sheep blood.

Ethanol Extracted Method

For each variety of plant, 50 mg of powder was taken, combined with 250 mL of 70% ethanol in a 1/5 ratio, and shaken for a full night. Filter paper suspension was used for filtration, and the resulting powder was then placed in a clean container to evaporate the alcohol (13).

Method Procedure for Wells

Pouring Columbia R medium into a Petri dish at a depth of 4 mm. expanding on plates to isolate bacteria using a cotton swab is H. pylori. After that, dry the plates for 30 minutes at 37 degrees Celsius and ensure that they are well-concentrated in a culture plate with multiple well-utilized antibiotics by using a sterile sterilization borer crock with a convenient width of 10 mm. The well was filled with a particular antibiotic. Plates should be incubated at 37°C to 18¯ 24hours. Measured the inhibition zone a rounded well (14).

4. RESULT AND DISCUSSION

In this work, plants extraction by ethanol for different components (G. glabra, P. granatum, and honey). Treated H. pylori bacteria with varying dilutions. The diameter measurement of the

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growth-inhibited zone and the concentration is tabulation that followed were used to assess the antibacterial activity of the plants using the agar well diffusion method.
The results showed in table No. 1 there is no effectiveness of G. glabra plant extract on H. pylori bacteria by inhibition zone diameter and p-value there is no significant variation and all so appear in figure no. (1).

Table (1): Inhibition Zone (Mm) Of Gycyrrhiza Glabra Plant Extract Against Helicobacter Pylori Bacteria

Concentration of extract (mg/ml)	**Plant extract**			**Mean of concentration**	**P-Value**
	1	2	3		
Amoxicillin 50mg/3ml	45 ± 0.0	42 ± 0.0	48 ± 0.0	45.00 A	0.94
Clarithromycin 100 mg/3ml	59 ± 0.0	43 ± 0.0	48 ± 0.0	50.53 B	0.96
Extract 50 mg/3ml	6.6 ± 0.0	5 ± 0.0	7.1 ± 0.0	6.23 C	0.23
Extract 25 mg/3ml	0 ± 0.0	4 ± 0.0	0 0.0	1.33 C	0.133
Extract 12.5 mg/3ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 C	0.00
Extract 6 mg/3ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 C	0.00
Extract 3 mg/3ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 C	0.00
Mean of extract Solvent	15.8 c	13.42 b	14.72 a	LSD0.05 Interference 0.6653	
LSD0.05 Solvent	2.304666				

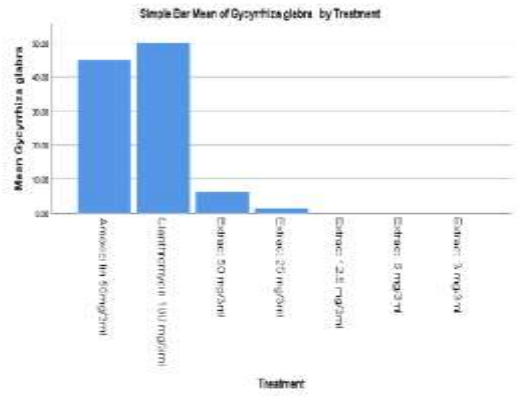

Figure (1) Effectiveness Of Gycyrrhiza Glabra Plant Extract Against Helicobacter Pylori Bacteria.

In table (2) showed effectiveness of P. granatum and G. glabra plants extracted against H. pylori appeared moderate effectiveness by inhibition zone diameter in concentration 50mg and 25mg with there is no significant variation in p-value and all so appear in figure no. (2).

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Table (2): Inhibition Zone (Mm) Of Punica Granatum and Gycyrrhiza Glabra Plant Extract Against Helicobacter Pylori Bacteria

Concentration of extract (mg/ml)	Plant extract			Mean of concentration	P-Value
	1	2	3		
Amoxicillin 50mg/3ml	45 ± 0.0	42 ± 0.0	48 ± 0.0	45.00 A	0.095
Clarithromycin 100 mg/3ml	59 ± 0.0	43 ± 0.0	48 ± 0.0	50.53 B	0.097
Extract 50 mg/3ml	12 ± 0.0	14.3 ± 0.0	10 ± 0.0	12.10 C	0.8
Extract 25 mg/3ml	7 ± 0.0	5 ± 0.0	4.4 ± 0.0	5.46 C	0.4
Extract 12.5 mg/3ml	3.2 ± 0.0	0 ± 0.0	1 ± 0.0	1.40 C	0.00
Extract 6 mg/3ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 C	0.00
Extract 3 mg/3ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 C	0.00
Mean of extract Solvent	18.02 c	14.90 b	15.91 a	LSD0.05 Interference 0.675803	
LSD0.05 Solvent	2.341051				

* The numbers refer to mean ± Standard error.
* Various vertically Significant changes are indicated by capital letters (P<0.05) between the concentrations.
*Various Horizontally Significant changes are indicated by small letters (p<0.05) between Solvents.

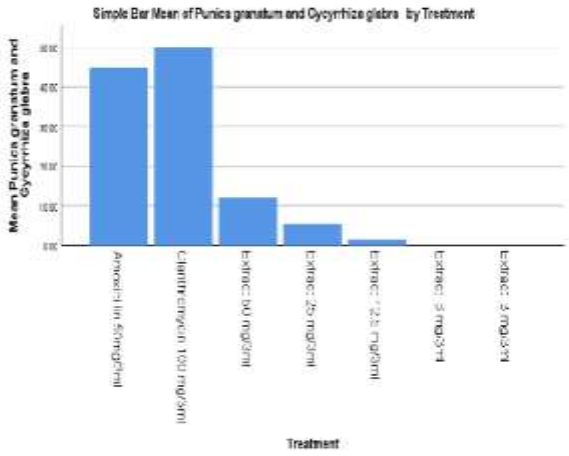

Figure (2) Effectiveness of Punica Granatum and Gycyrrhiza Glabra Plant Extract Against Helicobacter Pylori Bacteria

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The result showed in table no. (3) G. glabra and L. usitatissimium plant extract against H. pylori bacteria not effectiveness effect these appeared by diameter of inhibition zone and all so the P-value, figure no. (3) appear same results of mixed of plant extract.

Table (3): Inhibition Zone (Mm) Of Gycyrrhiza Glabra and Linum Usitatissimium Plant Extract Against Helicobacter Pylori Bacteria

Concentration of extract (mg/ml)	Inhibition zone			Mean of concentration	P-Value
	1	2	3		
Amoxicillin 50mg/3ml	45 ± 0.0	42 ± 0.0	48 ± 0.0	45.00 A	0.083
Clarithromycin 100 mg/3ml	59 ± 0.0	43 ± 0.0	48 ± 0.0	50.53 B	0.085
Extract 50 mg/3ml	6.7 ± 0.0	8 ± 0.0	7.3 ± 0.0	7.33 C	0.9
Extract 25 mg/3ml	3.8 ± 0.0	4 ± 0.0	4.1 0.0	3.96 C	0.53
Extract 12.5 mg/3ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 C	0.00
Extract 6 mg/3ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 C	0.00
Extract 3 mg/3ml	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 C	0.00
Mean of extract Solvent	16.35 c	13.85 b	15.34 a	LSD0.05 Interference 0.640285	
LSD0.05 Solvent	2.218012				

* The numbers refer to mean ± Standard error.
* Various vertically Significant changes are indicated by capital letters (P<0.05) between the concentrations.
*Various Horizontally Significant changes are indicated by small letters (p<0.05) between Solvents.

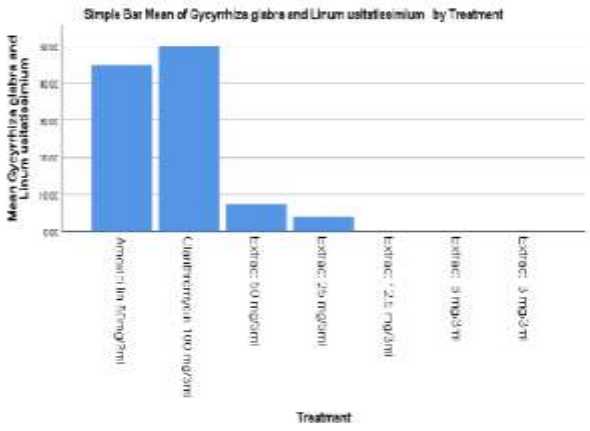

Figure (3) Effectiveness Of Gycyrrhiza Glabra And Linum Usitatissimium Plant Extract Against Helicobacter Pylori Bacteria.

In table no. (4) the results showed effectiveness inhibition zone(mm)of Honey and G. glabra against H. pylori bacteria in plant extract concentration (50mg, 25mg) in arrangement by

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inhibition zone of diameter and there is no significant variation in P-value, the figure no. (4) appear same effectiveness of table no. (4).

Table (4): Inhibition Zone (Mm) Of Honey and Gycyrrhiza Glabra against Helicobacter Pylori Bacteria

Concentration of extract (mg/ml)	Inhibition zone			Mean of concentration	P-Value
	1	2	3		
Amoxicillin 50mg/3ml	45 ± 0.0	42 ± 0.0	48 ± 0.0	45.00 A	0.083
Clarithromycin 100 mg/3ml	59 ± 0.0	43 ± 0.0	48 ± 0.0	50.53 B	0.085
Extract 50 mg/3ml	22 ± 0.0	17 ± 0.0	21 ± 0.0	20.00 C	0.025
Extract 25 mg/3ml	14.2 ± 0.0	16 ± 0.0	13 ± 0.0	14.66 C	0.01
Extract 12.5 mg/3ml	6 ± 0.0	4.8 ± 0.0	1.8 ± 0.0	4.6 C	0.4
Extract 6 mg/3ml	0 ± 0.0	0 ± 0.0	0.00	0.00 C	0.00
Extract 3 mg/3ml	0 ± 0.0	0 ± 0.0	0.00	0.00 C	0.00
Mean of extract Solvent	20.88 c	17.54 b	19.11 a	LSD0.05 Interference	
LSD0.05 Solvent	2.361442			0.681689	

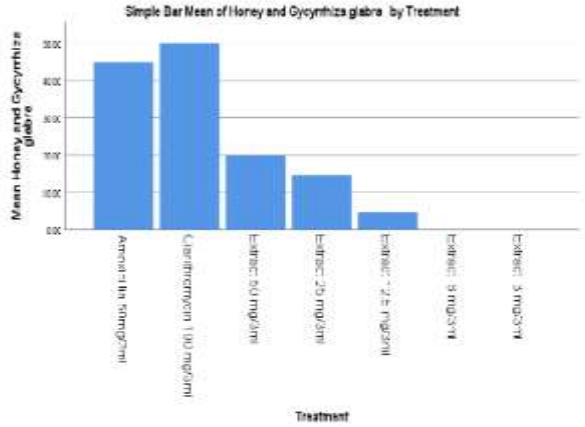

Figure (4) Effectiveness of Honey and Gycyrrhiza Glabra against Helicobacter Pylori Bacteria.

The results in table no. (5) shown there is effectiveness of mixed plant extract G.glabra, P. granatum and Honey in diameter of inhibition zone(mm) contra H. pylori bacteria for concentration (50mg, 25mgand 12.5mg) in arrangement with significant variation in p-value in concentration 50mg of the extract, and these results appear figure no. (5).

Table (5): Inhibition Zone (Mm) Mixed Of Gycyrrhiza Glabra, Punica Granatum and Honey Plant Extract against Helicobacter Pylori Bacteria

Concentration of extract (mg/ml)	Inhibition zone			Mean of concentration	P-Value
	1	2	3		
Amoxicillin 50mg/3ml	45 ± 0.0	42 ± 0.0	48 ± 0.0	45.00 A	0.09

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Clarithromycin 100 mg/3ml	59 ± 0.0	43 ± 0.0	48 ± 0.0	50.53 B	0.093
Extract 50 mg/3ml	26.3 ± 0.0	27.3 ± 0.0	25 ± 0.0	26.20 C	0.05
Extract 25 mg/3ml	14.3 ± 0.0	16.5 ± 0.0	13.8 ± 0.0	14.86 C	0.01
Extract 12.5 mg/3ml	10 ± 0.0	8.4 ± 0.0	6 ± 0.0	8.13 C	0.2
Extract 6 mg/3ml	4.9 ± 0.0	5 ± 0.0	6 ± 0.0	5.30 C	0.1
Extract 3 mg/3ml	0 ± 0.0	5 ± 0.0	0 ± 0.0	1.66 C	1.33
Mean of extract Solvent	22.78 c	21.02 b	20.97 a	LSD0.05 Interference	
LSD0.05 Solvent	2.435186			0.702978	

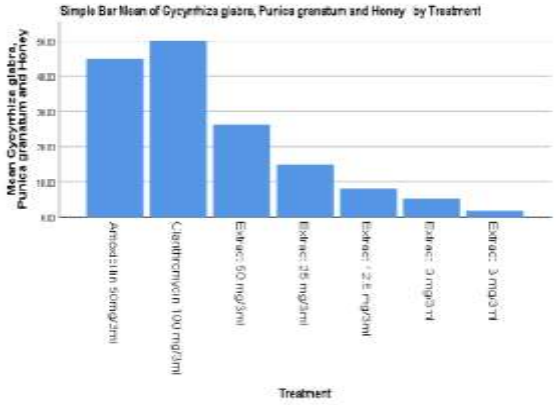

Figure (5) Effectiveness of Mixed Of Gycyrrhiza Glabra, Punica Granatum and Honey Plant Extract against Helicobacter Pylori Bacteria

5. DISCUSSION

It has been found that G. glabra, or licorice, has antibacterial action against a number of gram-positive and gram-negative bacterial strains, including H. pylori (38). Furthermore, the antiadhesive qualities of licorice also had positive effects against H. pylori (14). Licorice also showed anti-ulcer and anti-cancer properties, as well as clinical effects related to H. pylori infection. Licorice extract has been demonstrated to have anticancer properties in an in vitro study (16), while in vivo and clinical investigations have reported the therapeutic efficacy of deglycyrrhizinated licorice (DGL) on ulcers (15, 16). It has been demonstrated that G. glabra possesses anti-ulcerogenic qualities, which could be attributed to the cytoprotective mechanism of its antioxidant characteristics. These findings validated the traditional medicinal applications of licorice for the management of stomach ulcers (17).

These findings showed that treating patients with a complex mixture of L. paracasei HP7, which includes the extract of P. granatum and G. glabra, could inhibit the growth of H. pylori. As a result, this is a promising treatment for patients whose H. pylori infection is the cause of their stomach symptoms, such as gastritis (18). P. granatum is an old fruit that is eaten in large quantities worldwide. Voravuthikunchai and Mitchel's reported range for the minimum

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inhibitory concentration (MIC) of P. granatum crude extract against H. pylori (10 mg/ml) was met in the current investigation (19).

Inhibition zones from the ethyl acetate extract's effective fractions and subfractions were smaller than those from the P. granatum crude extract. This could be the result of various crude extract fractions and subfractions working in concert (20). H. pylori infection was completely eradicated after one week of in vivo administration of P. granatum peel extract and without the use of antibiotics. Evidently, using Peel extract resulted in a significant drop in colony count after one week of intervention ($p<0.05$). However, compared to CFU prior to treatment, antibiotic use was linked to a higher bacterial count; nonetheless, it considerably reduced H. pylori multiplication when compared to the non-treated group (21).

We showed that urease enzyme activity and H. pylori proliferation are significantly inhibited by pomegranate peel extract. To the best of our knowledge, this is the first publication revealing a potential method by which pomegranate peel extract inhibits H. pylori, by showing its potential inhibitory capabilities against urease enzyme activity. Using the disc diffusion method, similar results were obtained using pomegranate peel methanol extract, with an average inhibitory zone of 39 mm at 0.1 mg/disk (22). Moreover, greater MIC values ranging from 0.625 to 0.780 mg/mL were documented (23). The extract type, the variation in the H. pylori strains employed, and their susceptibility or resistance to antibiotics can be blamed for the discrepancies between our results and the values published in the literature factor (24).

The metronidazole half-life of this antibiotic was halved when PPEE was added. This implies that this antimicrobial combination may be used to provide effective therapy. Interestingly, a study by Voravuthikunchai et al. (23) found that by changing the hydrophobicity of the bacterium's cell surface, pomegranate fruit rind extract exhibits anti-adhesive effects against H. pylori to gastric mucosa. This finding may work in concert to help remove bacterial cells from the human body. Nevertheless, the mechanisms behind the synergistic effect of metronidazole and PPEE were not examined in our investigation. This is an important area for future research (25).

Due to its antibacterial properties, honey has been used to treat a variety of skin conditions.(26,27) All of the honey samples tested in our investigation had antibacterial activity against H. pylori, with the genuine Black Forest honey from Germany's Black Forest exhibiting the strongest antibacterial activity. The German honey known as Langnese follows this. Although the exact components of honey's antibacterial effect are unknown, propolis, flavonoids, flavones, tannins, and glucose oxidase and osmosis have all been linked to the polyphenolic substances present in honey. Plant nectars vary depending on the type of the plant, the time of year, and the plant's geographic location. These changes result in the product known as honey. According to Molan, honey's antibacterial properties stem from the presence of glucose oxidase, which, when diluted, releases hydrogen peroxide. In addition to promoting epithelial proliferation, the hydrogen peroxide thus released has antimicrobial properties (26, 31).

Burdon underlined that the usual pathway of wound healing involves the production of hydrogen peroxide as a result of injury or infection, which stimulates the proliferation of fibroblasts and epithelial cells. The antimicrobial properties of honey were attributed by Somerfield, Condon, and Osato to the osmotic action of its sugar content. Honey has a pH of 3.9 and comprises 38% fructose, 31% glucose, and 17% water. Due to its low water content, it

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soothes the wound and promotes wound healing through the hygroscopic absorption of water molecules on the surface of the wound. Our investigation confirms the findings of numerous previous studies that honey has antibacterial action against H. pylori in vitro (28, 29, and 30).

6. CONCLUSION

The study will used plant isolation as novel goods and medications. The results shown there is effectiveness of mixed plant extract G.glabra, P. granatum and Honey. The findings of this study have demonstrated the presence of antibacterial and antioxidant components in three plant extracts and honey.

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