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RESEARCH ARTICLE

Individual and Combined Antibacterial Activity of Honey and Medicinal Herbs against some common Clinical Pathogens

[*]C.R Shalinimol[1], C Asha Priya[1]

[1]PG Department of Microbiology, Scott Christian College, Nagercoil, Tamilnadu, India.

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ABSTRACT

In this present investigation, the individual and combined action of honey and five herbal extracts like ginger (Zingiber officinale), garlic (Allium sativum), liquorice (Glycyrrhiza glabra), purple fruited pea eggplant (Solanum trilobatum) and Indian Acalypha (Acalypha indica) were tested against five common clinical pathogenic bacteria such as Bacillus sp., Klesiella sp., Psudomonas sp., Streptococcus sp. and Staphylococcus aureus. All the bacterial isolates were less susceptible to honey and the plants individually. But both honey and the herbs combined together and played a good role against the pathogens. Garlic with honey acted more against S. aureus (22mm), Bacillus sp. (20mm), Klebsiella sp. (16mm) and Streptococcus sp. (12mm). Ginger with honey was highly active against Pseudomonas sp. (18mm) and Streptococcus sp. (14mm). Liquorice with honey was active against Pseudomonas (19mm) and Streptococcus sp. (13mm) and purple fruited pea eggplant with honey was highly effective against Streptococcus sp. (17mm). Acalypha did not play a good role against these pathogens but Streptococcus with 9mm and Klebsiella with 8mm zone could produce a suitable action. All the tested bacteria were sensitive to a mixture of honey and garlic and all the plants with honey could inhibit the growth of Streptococcus sp. In the case of minimal inhibitory concentration, 1.8 to 2ml of the mixture of the plant extracts and honey made a complete destruction of the pathogens. All such outcomes in total, exhibit the possible applications of these medicinal herbs combined with honey against many bacterial diseases.

Keywords: Honey, Zingiber officinale, Allium sativum, Glycyrrhiza glabra, Solanum trilobatum, Acalypha indica.

1. INTRODUCTION

Honey is a sugary nourishment made by honey bees by consuming nectar sucked from flowers. The honey bees take up the process of regurgitation and evaporation to obtain honey from the nectar. The honey is in fact collected to be stored as a chief food source. It is stored in honey combs that are made of wax in beehives. Honey comprises not less than 200 substances and the chief substances are sugar and water. It likewise contains minerals, proteins, free amino acids, catalysts, vitamins, natural acids, flavonoids, phenolic acids, and different phytochemicals [1]. The synthesis of honey fundamentally relies upon the flower sources; in any case, definite external variables assume a part. Examples of such variables are regular and natural factors and handling strategies. Honey finds itself important for the treatment of coronary illness, cancer, cataracts, and a few inflammatory infections. The remedial activities of honey incorporate antioxidant and antimicrobial properties, and honey is found to be effective at healing wounds and involves in better anti-inflammatory actions [2]. Anti-oxidation is one among the honey's organic properties. The presence of enzymatic antioxidants (glucose oxidase, catalase) and non-

*Corresponding author. Tel.: +919487686711

Email address: shalinimol_2008@yahoo.com (C.R.Shalinimol)

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enzymatic antioxidants (flavonoids, ascorbic acid and phenolic acids) has been detected in many honeys [3]. Antibacterial properties have also been described for honeys produced in different places. The antimicrobial action of honey is due to several parameters, including osmolarity, pH and the production of H_2O_2 and other chemical compounds [4]. The antibiotic properties of several varieties of honey against human pathogenic bacteria such as Staphylococcus aureus, Enterococcus faecalis, Pseudomona aeruginosa, Escherichia coli, Morganella morganii and Klebsiella pneumonia were studied. Plants are fundamentally the chief foundation stone for medicine. It is proved that medicinal plants exhibit high therapeutic values as they possess high levels of secondary metabolites. In additional to having less cost, the medicinal plants enjoy efficacy and availability all over the world. The main benefits of using medicinal plants are that they are used for several treatments and they are safe as well [5-8].

Ginger (Zingiber officinale) being a domestic plant is acceptable as a good remedy world-wide that includes India and parts of ancient china. These places have roots that are fresh and dry and are regarded to have unique medicinal value. Ginger as such is used as remedial actions for infections that are caused because of cold, nausea, asthma, cough, colic, heart palpitation, swelling, dyspepsia, loss of appetite, and rheumatism [9].

Garlic (Allium sativum) involves an extensive ability to treat cold, cough and asthma, and is found useful in strengthening the body's immune system [10]. Additionally garlic is utilized all over to cure Alzheimer's disease, cancer, cardiovascular diseases that incorporate atherosclerosis, strokes, hypertension, thrombosis, hyperlipidemias and infections. It also improves children's conditions and are used for dermatologic applications and in diminishing stress [11]. Normally garlic is well known to possess anti-bacterial, anti-fungal, anti-cancer and anti-viral properties [12] and the primary anti-microbial ingredient of garlic is found to be oxygenated sulphur compound, thio-2-propene-1-sulfinic acid S-allyl ester, which is known as allicin [13].

Glycyrrhiza glabra (Liquorice) being a sweetened, moist herb is soothing and exhibits inflammatory and medicinal values. Besides cleansing the liver, it also safeguards it. As it possess medicinal values it is utilized for Addison's disease, asthma, bronchitis, coughs, peptic ulcer, and arthritis [14]. Anti-microbial actions have been identified in roots and rhizomes as per investigations conducted so far. However there is also some information regarding the medicinal value of leaves of liquorice against microorganisms [15].

Solanum trilobatum (purple fruited pea eggplant) is a thorny creeper with bluish white flower and grows as a climber under shrub. It is one among the plants that possess medicinal value being widely accessible in the southern parts of India. This plant has been made use in herbal medication to cure several types of respiratory ailments like bronchial asthma and tuberculosis [16].

Acalypha indica also called as Kuppaimeni in Tamil, is an yearly weed generally present in various parts of Asia, belonging to the family Euphorbiaceae. The leaves, root, stalk and flowers of the plant possess anti-microbial activity. Such an activity of Acalypha indica plant was because of the incidence of phyto chemical compounds such as alkaloids, tannins, saponins, steroids and proteins. The main phytochemical ingredients of the plant are alkaloids like acalypus and acalyphine and this plant is utilized as a diuretic, antihelmintic and as a remedy for respiratory ailments like bronchitis, asthma and pneumonia [17].

The current examination revealed the individual and combined consequence of the above mentioned five medicinal plants with honey. They were tested with five different common human pathogenic bacteria for their antimicrobial activities.

2. MATERIALS AND METHODS

2.1. Isolation of microbes

The bacterial cultures like Klebsiella sp., Pseudomonas sp., Streptococcus sp. and Bacillus sp. were isolated by serial dilution technique in the laboratory from the hospital sewage samples. The isolated microbes were recognized by the

aid of morphological studies and other biochemical examinations according to standard means and the results were compared with the information in Bergey's manual of systemic bacteriology. Staphylococcus aureus was purchased from Vivek clinical Laboratory, Nagercoil, Tamil Nadu.

2.2. Collection of herbal medicines

The medicinal herbs were collected from different fields as well as from the Ayurvedic medical shops from Nagercoil, Kanyakumari District, Tamil Nadu. The common and botanical names of the plants and parts of the useful plants are indicated in Table A1.

2.2.1. Extraction

The useful part of the medicinal plants (ginger, garlic, liquorice, purple fruited pea eggplant and Indian Acalypha) were cleansed using distilled water that is sterilized before weighing. 10 grams of each plant parts were macerated individually using mortar and pestle with 10ml of honey. The plant parts of 10 grams were also macerated individually with 10ml of distilled water (aqueous extract) for checking their activities alone. Conical flask was used in the process of filtration with the aid of Whatman's filter paper number 4. The filtrate thus obtained was used for further antimicrobial testing.

2.2.2. Antibiotic sensitivity test

The herbal medicines like garlic, ginger, liquorice, purple fruited pea eggplant and Acalypha were tested for their antibiotic activity with and without honey as well as only honey (without plant combination) against different pathogenic bacteria like Klebsiella sp., Staphylococcus aureus, Pseudomonas sp., Streptococcus sp. and Bacillus sp., and they were tested by the help of Agar well diffusion method in the Muller Hinton Agar plates. Sterile swab was used to spread the culture on top of agar plates, and wells that comprise 6mm diameter were created on the surfaces of agar. All the above plant extracts with and without honey of 50µl each was added to the respective wells, and the plates were incubated at 37°C for 24 hours. Control plates were also maintained for each bacterium.

2.2.3. Minimal inhibitory concentration

The test organisms were maintained as broth culture (bacterial suspension). In each test tube, 10ml of culture broth was maintained. Various concentrations (0.2 to 2ml) of plant extracts with honey and without honey were added using sterile tips to the respective over night culture tubes and incubate the tubes for the time duration of 24 hours at 37°C in a shaker to ensure the inhibitory level. The suspensions of all the five bacteria act as control.

3. RESULTS AND DISCUSSION

3.1. Well diffusion test

The plant extracts could form zones in some culture plates even in small amount (50 micro litres) which is exhibited under table A2. The individual action of honey was more against Streptococcus and this is shown in table A3. The plant extracts together with honey can produce a good change in zone formation. This is presented under table A4 and figure A1. The plant extracts like garlic, liquorice and purple fruited pea eggplant with honey individually can kill more number of pathogens. From this observation we can noticed that the honey can act as a good antibiotic agent with some medicinal plants.

3.1.2. Solution assay (MIC)

This test was carried out by the calorimetric method. The mixture of honey and plant extracts as well as honey and medicinal extract alone treated liquid cultures were poured into the cuvettes and the absorbance (optical density) readings were taken at the wave length of 600nm in the calorimeter. The plain nutrient broth acted as a blank. The liquid cultures alone acted as controls, which are shown under table A5. The OD values of 0.2 to 1.0ml were given in the tables A6 and A7.

This study is a preliminary evaluation of antimicrobial activity of honey with five different herbal medicines.

In our experiment, we observed the individual effect of honey and the herbal medicines as well as the combined activity of honey with medicinal herbs against five different gram positive and negative bacterial cultures.

Plants are regarded of having favourable medicinal response for diseases and are reported

in conventional Indian system of medicine. Researchers from various sects of the world have investigated the action of plant extracts on microbes. A report was concluded that plants possess antibacterial, antifungal and other activities [18-20].

An experiment with honey against five gram negative bacteria (E.coli, Shigella flexneri, Salmonella enterica serovar Typhimurium, Klebsiella pneumoniae, Pseudomonas aeruginosa) [21] and three gram positive bacteria (Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes) was conducted.

In the present study, well diffusion method and minimum inhibitory concentration assay are used to determine the antibacterial effects of honey with different medicinal plant extracts against five common pathogenic bacteria.

Several studies by well diffusion method to find out antimicrobial action exhibited by honey were conducted [22]. Anti-microbial active honey together with medicinal plants produced a growth inhibition around the well containing honey and medicinal plant extract samples. Our results show that honey alone could produce only small inhibition zones against the pathogens than with the herbal extracts.

This study revealed the zone of inhibitions to a maxim by themixture of honey and ginger against Pseudomonas (18mm) and Streptococcus (14mm). Bacillus and Klebsiella would produce only 8mm of zone. There was no zone at all in Staphylococcus plate. The issues that pave way for ginger to possess high susceptibility towards test organisms are not precisely known but it may be due to the secondary metabolite (inhibins) and phytochemicals (gingerol and shagalol, flavonoids) [23]. The outcomes obtained from this study approves with that of [24-28].

Garlic (Allium sativum) has been used as a remedy in several cultures since the time of Egyptian pyramids. For over thousands of years, garlic extract has been utilized for treatment of infections [29]. The Aqueous Garlic Extract (AGE) that possesses anti-bacterial effects combats 17 multidrug-resistant gram-positive and gram-negative bacterial segregates, that includes Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli and Proteus sp., The antibacterial actions that possess varied concentration of AGE by involving well diffusion process was categorized based on inhibition zones of 15 gram-positive and two gram-negative pathogenic bacteria [30]. In the research, the combination of honey with garlic exhibited a worth action against all the five pathogens. But S. Aureus (22mm), Bacillus sp. (20mm) and Klebsiella sp. (16mm) showed big inhibition zones.

The inhibition zone of mixture of Apis mellipodae as well as extract of garlic against Salmonella NCTC 8385, S. Aureus (ATCC 25923) and S. Pneumonia (ATCC 63) were considerably better (nearly 30-35mm) [30].

Liquorice is the generally utilized medication and agent that adds flavour in Kampo medicines. Kampo medicines are the medicines of Japan that were modified from traditional Chinese medicine. The characters of pharmaceutical include anti-bacterial, anti-viral, anti-inflammatory and anti-carcinogenic properties [31]. The extract of liquorice that has flavonoid content is a strong inhibitor of oxygen consumption in bacterial cells. In this research, liquorice exposed anti-bacterial action against Klebsiella (gram negative) and Streptococcus sp.,(gram positive) bacteria together with honey than their distinct action. Liquorice at a concentration of 50 per cent has an inhibitory action on Streptococcus mutants [32].

Traditionally, Solanum trilobatum Linn (ST) has been made use in various parts of the Indian country to treat various diseases [33]. The bitter roots of this plant are utilized for being consumed in the form of electuary, decoction or powder. The berries and flowers are used for treating cough. The parts of the plant in the form of decoction are utilized for curing bronchitis [34-36]. The alkaloids stated in the genus of Solanum comprise solanine, solasonine and solamarine [37]. The chief alkaloids recognized in the extract of ST that is alcoholic are β-solamarine [38-42]. Phytochemical screening of the alcoholic extract of ST has recognized the incidence of a base similar to that available in Solanum dulcamara possessing antimitotic and anti-fungal properties [38]. Perhaps because of the incidence of these alkaloides, Solanum trilobatum acquires its antibacterial property. In

this research, it exhibited better antibacterial action in Streptococcus lawn (17mm). Bacillus (5mm) and Klebsiella (8mm) was also subdued in a meager amount. These activities did not occur separately, but were done in combination of honey [39]. It was also documented that by biological means the silver Nano particles were synthesized. The fresh fruits of Solanum trilobatum possessed the antibacterial action opposing gram positive Streptococcus mutants and gram negative Klebsiella pneumonia.

Acalyphaindica is widely made use as traditional medicine in different countries. The leaves of A. Indica have acalyphine which is utilized to treat sore gums and has a post-cotical and has effects exhibiting fertility, anti-inflammatory, diuretic and other such properties [40].

In our study we obtained honey extract assayed for the antibacterial activity against five common bacterial pathogens. Acalypha-honey mixture showed good activity against Streptococcus (9mm) and Klebsiella sp. (7mm) compared to other organisms. The acetone extract of Acalypha indica produced the inhibition zone of 16mm for Klebsiella sp. [41].

The solution assay also showed gradual decrease in the concentration of bacteria when the concentration of honey and medicinal herbs extract become increased. Its complete cell death took place nearly when the concentration of the mixture of honey and herbal extract became 1.8 to 2ml. These entire outcomes approve with the outcome of [42-47].

4. CONCLUSION

The obtained results in this study validated that the honey and the tested five medicinal herbs have antibacterial potency which is able to establish valuable inhibition zones in-vitro. All the five tested organisms are susceptible for mixture of honey and garlic. The growth of Streptococcus sp. was inhibited by all the tested plants with honey. The solution assay also showed the decrease in the growth of organisms with increase in the concentration of plants extract and honey. The activity of the mixture of honey with medicinal plants was more than the activity of the honey and the medicinal plants alone. This is how we can suggest that honey together with medicinal herbs could be used for curing many of the bacterial diseases.

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APPENDIX A

Table A1.Common and botanical names and their useful parts of herbs

Sl. No.	Common name	Botanical name	Used part of plants
1.	Garlic	Allium sativum	Bulb
2.	Ginger	Zingiber officinale	Rhizome
3.	Liquorice	Glycyrrhiza glabra	Root
4.	Purple fruited pea eggplant	Solanum trilobatum	Whole plant
5.	Indian acalypha	Acalypha indica	Leaf

Table A2.Inhibition zone produced only by herbs against five pathogens

Name of the herbal extract	Zone diameter (milli metre)				
	Bacillus	Klebsiella	Staphaylococcusaureus	Streptococcus	Pseudomonas
Ginger	3 mm	No zone	No zone	2 mm	No zone
Garlic	2 mm	2 mm	3 mm	2 mm	5 mm
Acalypha	No zone	2 mm	No zone	4 mm	3mm
Liquorice	2 mm	No zone	No zone	3 mm	2mm
Purple fruited-pea Eggplant	1 mm	4 mm	No zone	3 mm	No zone

Table A3.Inhibition zone produced only by honey against five pathogens

Sl. No	Names of the bacteria	Zone diameter
1	Bacillus	2 mm
2	Klebsiella sp.	3 mm
3	Staphylococcus aureus	3 mm
4	Streptococcus sp.	11 mm
5	Pseudomonas sp.	5 mm

Table A4.Inhibition zone produced by the mixture of honey and herbal extracts

Name of the herbal extract with honey	Zone diameter (milli metre)				
	Bacillus	Klebsiella	Staphylococcusaureus	Streptococcus	Pseudomonas
Ginger	8 mm	8 mm	No zone	14 mm	18 mm
Garlic	20 mm	16 mm	22 mm	12 mm	9 mm
Acalypha	6 mm	8 mm	4 mm	9 mm	5 mm
Liquorice	4 mm	8 mm	3mm	13 mm	19 mm
Purple fruited-pea Eggplant	5 mm	8 mm	3 mm	17 mm	2 mm

Table A5.OD values of bacterial suspension

Sl. No.	Names of the bacteria	OD values at 600 nm
1	Bacillus	1.44
2	Streptococcus sp.	1.39
3	Staphylococcus aureus	1.34
4	Klebsiella sp.	1.38
5	Pseudomonas sp.	1.53

Table A6.MIC of herbal extracts against pathogenic bacteria

Name of the bacteria	Name of the herbs with honey	O.D values of the herbal extract treated culture (600nm)				
		Concentration of herbs with honey extracts (ml)				
		0.2	0.4	0.6	0.8	1.0
Bacillus sp.	Ginger	1.15	1.12	1.08	1.04	0.98
	Garlic	1.14	1.06	0.84	0.76	0.67
	Acalypha	1.39	1.33	1.28	1.22	1.14
	Pea eggplant	1.29	1.16	0.93	0.87	0.82
	Liquorice	1.25	1.16	1.09	1.03	0.87
Streptococcus sp.	Ginger	1.25	1.21	1.18	1.12	0.96
	Garlic	1.33	1.24	1.19	1.15	0.83
	Acalypha	1.26	1.22	1.16	1.09	1.03
	Pea eggplant	1.35	1.30	1.28	1.17	1.13
	Liquorice	1.43	1.33	1.25	1.15	0.91
Staphylococcus aureus	Ginger	1.33	1.28	1.24	1.19	1.17
	Garlic	1.01	0.79	0.74	0.70	0.64
	Acalypha	0.97	0.95	0.92	0.84	0.73
	Pea eggplant	1.13	1.08	1.03	0.99	0.92
	Liquorice	0.93	0.87	0.84	0.80	0.74
Klebsiella sp.	Ginger	1.11	1.04	1.01	0.97	0.92
	Garlic	1.21	1.19	1.15	1.13	1.08
	Acalypha	1.06	1.03	0.99	0.95	0.91
	Pea eggplant	1.07	1.04	0.99	0.93	0.90
	Liquorice	1.13	1.09	1.05	1.03	1.01
Pseudomonas sp.	Ginger	0.91	0.88	0.85	0.81	0.79
	Garlic	0.85	0.82	0.78	0.70	0.62
	Acalypha	1.25	1.22	1.14	1.05	1.00
	Pea eggplant	1.20	1.13	1.09	1.04	1.00
	Liquorice	1.09	1.07	1.05	1.02	0.97

Table A7.MIC of mixture of herbal extracts and honey against pathogenic bacteria

Name of the bacteria	Name of the herbs	O.D values of the mixture of honey and herbal extracts treated culture (600 nm)				
		Concentration of herb extracts (ml)				
		0.2	0.4	0.6	0.8	1.0
Bacillus sp.	Ginger	0.80	0.73	0.65	0.63	0.52
	Garlic	1.04	0.75	0.67	0.64	0.59
	Acalypha	1.32	1.25	1.09	0.96	0.84
	Pea eggplant	0.98	0.92	0.89	0.87	0.85
	Liquorice	1.01	0.98	0.97	0.94	0.74
Streptococcus sp.	Ginger	0.99	0.86	0.84	0.75	0.73
	Garlic	1.28	1.19	1.15	1.12	0.96
	Acalypha	1.20	1.19	1.11	1.07	0.61
	Pea eggplant	1.30	1.18	1.16	1.08	0.94
	Liquorice	1.06	1.02	0.96	0.85	0.81
Staphylococcus aureus	Ginger	1.21	1.10	1.08	1.01	0.92
	Garlic	1.13	1.09	1.06	1.00	0.94
	Acalypha	1.01	0.97	0.96	0.95	0.89
	Pea eggplant	1.01	0.98	0.95	0.94	0.88
	Liquorice	1.10	1.05	1.00	0.99	0.95
Klebsiella sp.	Ginger	0.91	0.89	0.88	0.87	0.86
	Garlic	1.24	1.17	0.98	0.94	0.90
	Acalypha	0.93	0.87	0.83	0.76	0.71
	Pea eggplant	0.73	0.69	0.61	0.59	0.58
	Liquorice	1.01	0.88	0.70	0.68	0.65
Pseudomonas sp.	Ginger	1.04	0.82	0.78	0.77	0.75
	Garlic	0.75	0.73	0.71	0.70	0.62
	Acalypha	1.24	1.19	1.15	1.11	0.86
	Pea eggplant	1.09	1.01	0.85	0.78	0.72
	Liquorice	1.45	1.23	1.12	0.94	0.81

APPENDIX B

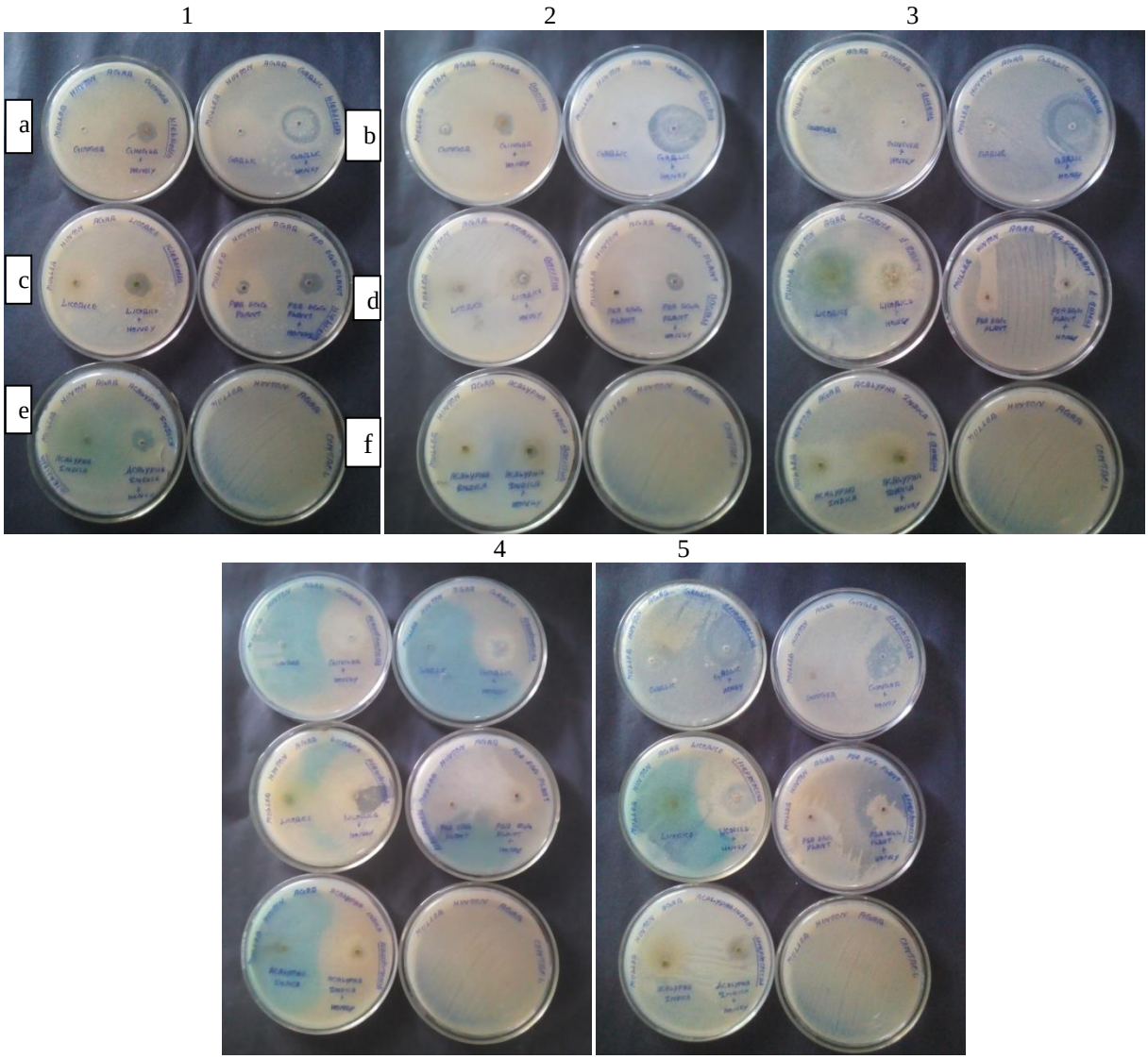

Figure B1.Inhibition of bacterial growth with 5 different herbal extracts and mixture of honey with herbal extracts against 1) Klebsiella 2) Bacillus 3) Staphylococcus 4) Pseudomonas 5) Streptococcus and inside the figure a) Ginger b) Garlic c) Liquorice d) purple fruited pea eggplant e) Acalypha f) Control