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Bacterial Examination in Spoiled Vegetables, Its Isolation, Identification and Antibacterial Activity of Turmeric, Pongamia and Betel Leaf

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ABSTRACT: Microbial contamination is a major concern in the food and pharmaceutical industries, leading to economic losses and public health risks. Natural products such as turmeric, betel leaf, and Pongamia leaves have been traditionally used for their antimicrobial properties. This thesis aimed to examine, identify, and isolate bacteria from selected spoiled vegetable samples and to evaluate the antibacterial activity of turmeric, betel leaf and Pongamia leaves. Bacterial isolation was performed using standard microbiological techniques, and the identification was done based on cultural characteristics. The antibacterial activity was assessed using disc diffusion method. The results showed the presence of bacterial contaminants in the tested samples, including Staphylococcus sp. And Escherichia coli. The turmeric, betel leaf, and Pongamia leaves extracts exhibited significant antibacterial activity against the tested bacteria. The phytochemical analysis of the extracts revealed the presence of alkaloids, flavonoids, phenols, tannins, and saponins, which may contribute to their antibacterial properties. This research provides valuable insights into the microbial contamination of food and pharmaceutical samples and the potential of natural products as antimicrobial agents. The findings of this study can contribute to the development of new antimicrobial agents based on natural products and the improvement of food and pharmaceutical safety.

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KEYWORDS: Vegetables, Spoilage, Turmeric, Pongamia pinnata, Betel leaf, Antibacterial activity, Phytochemical analysis

INTRODUCTION

Fruits and vegetables are important components of a healthy and balanced diet. Their sufficient daily consumption could help prevent major diseases such as cardiovascular diseases and certain cancers. According to World Health Organisation / Food and Agriculture Organisation (WHO/FAO) report published in 2004, a minimum of 400 g of fruits and vegetables per day, excluding potatoes and other starchy tubers, are recommended for the prevention of chronic diseases such as heart disease, cancer, diabetes and obesity, as well as for the prevention and release of several micronutrient deficiencies.

Most of our foods are excellent source for rapid microbial growth. Food materials contain organic substances in plenty and sufficient amount of water, and they may be either neutral or slightly acidic in nature. They are subjected to natural contamination by many different kinds of microorganisms, including pathogens. The microbial contamination has various reasons such as contact of vegetables with soil, dust, water and also due to rough handling during harvest time, marketing condition, during storage and also condition after purchased by the customers.The different types of microorganisms can be seen including plant and human pathogens. They will cause spoilage of vegetables. The spoilage in vegetables shows the softening of tissues. The unpleasant odour and flavours develop. Hence spoilage of fruits will make them unpalatable and undesirable for human consumption.

Betel leaf

In many countries, medicinal plants are being used as alternative to treat wounds. The leaf is the most widely used and studied part of the betel vine. It contains numerous phytochemicals such as alkaloids, tannins, glycosides, reducing sugars, phenolic compounds, flavonoids, steroids, terpenoids and saponins were found.

Turmeric

Curcuma longa is a medicinal plant that botanically is related to *Zingiberaceae* family. *C. longa*, commonly known as **'turmeric'**, is widely used as a spice and colouring agent, and is well known for itsmedicinal properties. Components of turmeric

are named curcuminoids, which include mainly curcumin (diferuloyl methane), dimethoxycurcumin, and bisdemethoxycurcumin. Curcumin is the most important fraction which is responsible for the biological activities of turmeric.

Pongamia pinneta

This multi-purpose tree is also used as insect repellent, antiseptic etc. The plant is known to have several medicinal properties such asanticonvulsant activity, neuroprotective activity, gastroprotectiveactivity, anthelminthic activity, Antiinflammatory activity, antinociceptive activity, antidiabetic activity, anti-lice activity, antihyperglycemicactivity, larvicidalactivity, antiviral activity, sunscreen activity, anti-arthritic activity, antioxidant activity, antibacterial activity etc.

Microbial analysis is the valuable way of evaluating the emerging risk that concern both the monitoring authorities and food consumers as well. Disinfectant wash is essential to reduce fresh vegetable microbial loads. There are several strategies such as physical and chemical treatments, which have been studied to decontaminate fresh cut vegetables.

MATERIALS AND METHODS

Five types of unwashed and unprocessed spoiled vegetables comprising of Carrot, Pumpkin, Potato, Chayote and Brinjal were collected in plastic bag from vegetable shop of Nazareth. Bacterial samples were plated in Nutrient Agar Medium and incubated at 37°C in Laminar Airflow Chamber.After 24 and 48 hours of incubation, the bacterial growth was observed. They were counted and tabulated. Further the Morphological analysis was conducted to determine different bacterial colonies and to observe morphological variation of bacterial growth. Gram staining was conducted to study the cellular morphology of isolated bacteria.

ISOLATION OF BACTERIA

The bacteria were isolated from spoiled vegetables by using serial dilution agar plate method. The spoiled vegetables were crushed into presterilized mortor and pestle with distilled water to from suspension, which was serially diluted from 10-1 to 10-5 dilutions.

Antimicrobial activity of Plant materials

Fresh leaves of Piper betle, Pongamia spp, and rhizome of Curcuma longa were collected from the plants. The leaves and the rhizome were chopped into small pieces and they were air dried for almost 7 days. The dried parts were blended to powder to increase the surface area for extraction in a blender. The entire weight of leaf powder was 20g. Cold extraction was carried out by soaking the powdered leaves for 72h in an enclosed glass jar with the solvent. The solvent used for extraction is ethanol. The solvent is separated from the extract by evaporation. Antibacterial activity was carried out using Agar well diffusion method. Zone of inhibition will be formed and their measurements were noted in millimetres and the results are interpreted.

RESULT AND DISCUSSION

In this study, various bacteria were isolated and identified from 5 different Spoiled vegetables such as Brinjal, potato, carrot, pumpkin and chayote which were collected from Nazareth. The isolation of bacteria has been done by pour and streak plate methods in Nutrient agar using 5 different samples of spoiled vegetables at different concentrations. The culture of bacteria in various spoiled vegetable samples was observed after 24 and 48 hours and the total bacterial count is done using Colony counter. The different concentrations showed varied bacterial growth.As numerous bacterial growth were observed in 10-2 concentration which is not feasible for counting.The graph 1 (Plotted for Bacterial growth in 10-5 concentration of Nutrient Agar) shows that the highest bacterial count was observed in Potato in both 24 and 48 hours in view of Nutrient Agar. According to classical bacteriology, most species of bacterial isolate can be differentiated based on Simple Gram Staining Technique. Gram Positive Bacteria were found in Potato, Pumpkin and Chayote and Gram Negative Bacteria have been found in Brinjal, Potato, Carrot and Chayote. Based on the morphology such as shape, size, texture, arrangement and colour of bacterial isolates they have been grouped due to their similarity. In Table 1, the morphology of bacteria colony has been observed and was noted. In Table 2, the shape and arrangement of bacteria were observed and they were recorded. From all these data (Stain type, Colony Morphology and Size and arrangement).The bacteria have been predicted. In Brinjal sample, bacteria observed were E.coli. The Potato sample was observed with E.coli and Staphylococcus sp. E.coli has been found in Carrot. The bacteria observed in Pumpkin sample was Staphylococcus sp. And the Chayote sample was found to be observed with E.coli and Bacillus sp.Antibacterial activity of turmeric extract, Pongamia leaf extract and betel leaf extract were done with agar well diffusion method. From these data, different concentrations of extracts show different zones of inhibition. The turmeric show maximum zone of inhibition (average 13.75mm) in higher concentration. Pongamia leaf extract showed maximum zone of inhibition (average 14mm). From the data, the maximum zone of inhibition (average 18.5mm) in higher concentration of Betel leaf extracts (Table 4, 5, 6)(Fig. 5, 6, 7).The Phytochemical analysis of three plant extracts was done in the laboratory. The result table shows that the turmeric extract contains Alkaloids, Terpenoids, Xanthoproteins, Phenolic compounds, Cardiac glycosides and Saponins. The Pongamia leaf extract contains Alkaloids, Saponins, Cardiac glycosides and Phenolic compounds. The Phytochemical analysis of Betel leaf extract showed that it contains Saponins, Terpenoids, Phenolic compounds and Aromatic acids.

Table 1. Morphology of Bacterial Colony

S. No.	Sample	Morphology of colony
1	Brinjal	Whitish, Round, Flat, large, Smooth and opaque
2	Potato	Creamy white, Circular large, Opaque Whitish, Flat, Round, Smooth
3	Carrot	Whitish, circular large, opaque, Flat Reddish, Irregular, Raised, Opaque.
4	Pumpkin	Reddish, Circular, large, flat Whitish, round, flat, opaque, Creamy white, filamentous, raised
5	Chayote	Whitish, flat, opaque, circular Reddish yellow, irregular, opaque.

Table 2 Identification of Bacteria

S. No.	Sample	Gram type	Shape and arrangement	Bacteria predicted
1	Brinjal	Negative	Small rods	*E.coli*
2	Potato	Negative Positive	Small rods Cocci in bunches	*E.coli Staphylococcus sp.,*
3	Carrot	Negative	Small rod	*E.coli*
4	Pumpkin	Positive	Cocci in bunches	*Staphylococcus sp.,*
5	Chayote	Positive Negative	Rods in chain Short rods	*Bacillus sp., E.coli*

Fig.1: BACTERIAL GROWTH ON NUTRIENT AGAR IN 24 HOURS.

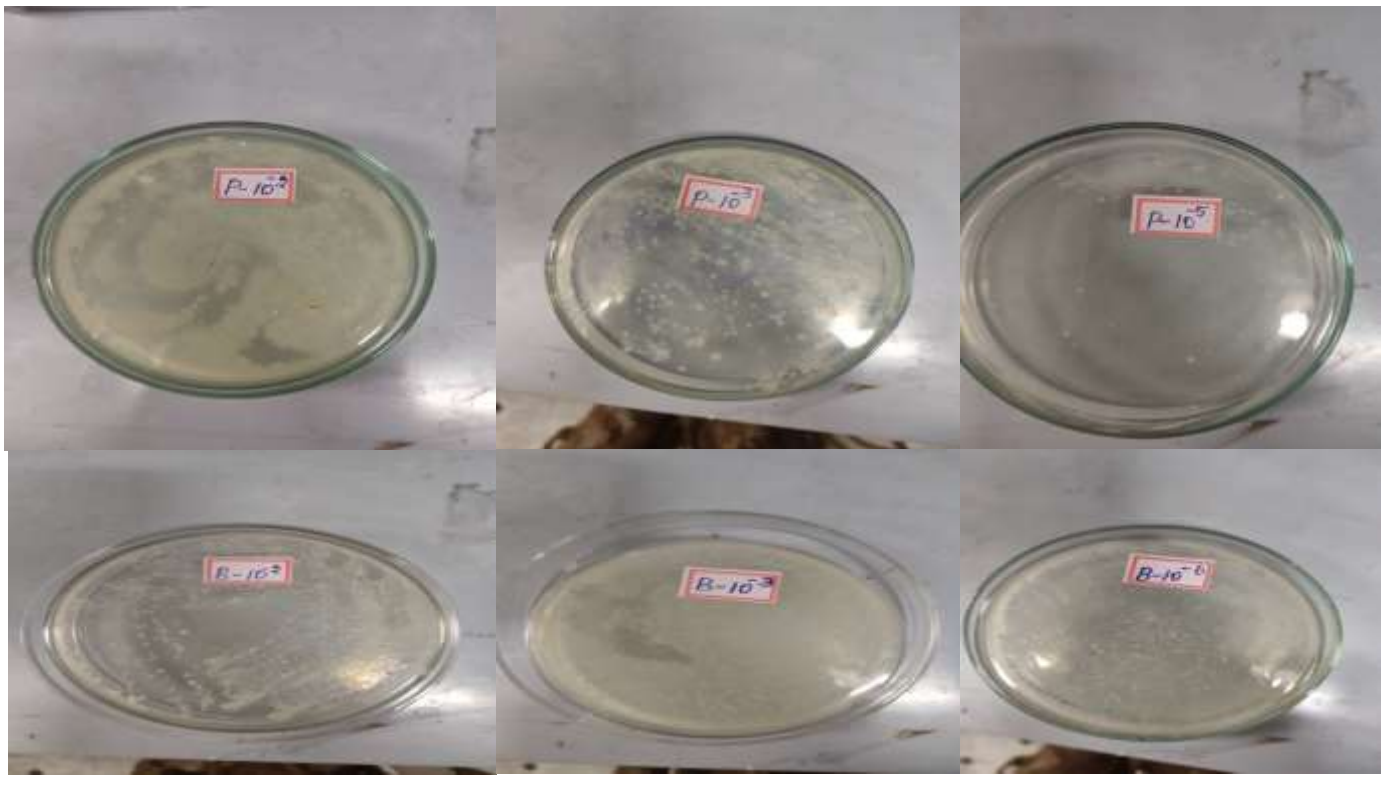

FIG-2: BACTERIAL GROWTH ON NUTRIENT AGAR IN 48 HOURS

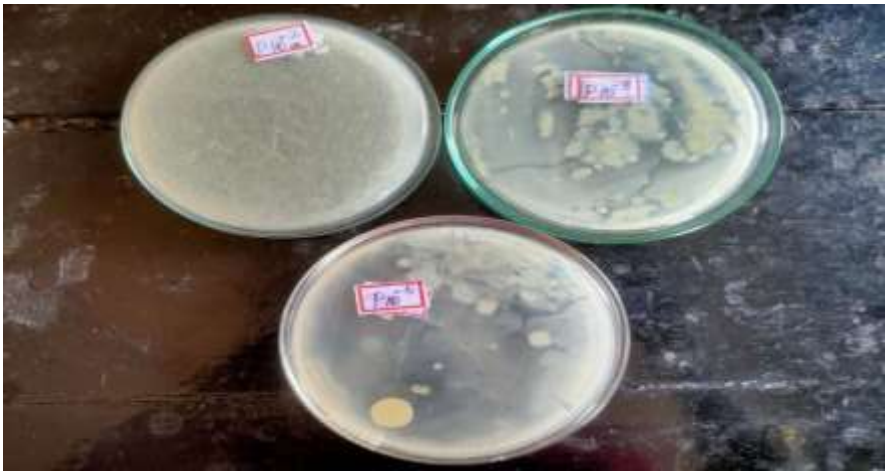

FIG-3: Gram positive bacteria from pumpkin

FIG-4: Gram positive and Gram negative bacteria from potato

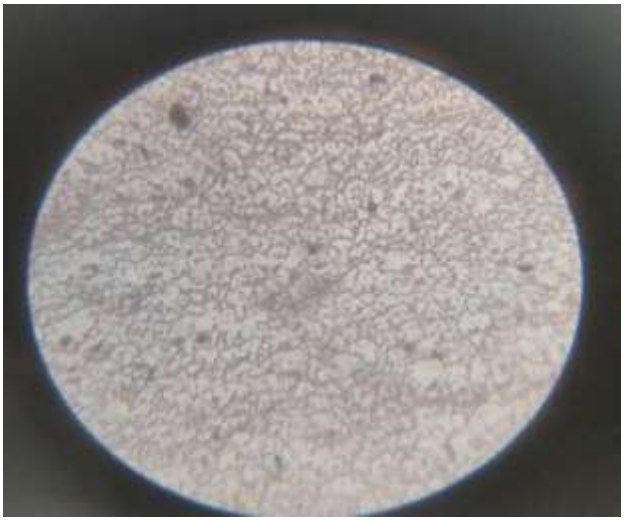

FIG.5: Gram negative bacteria from carrot

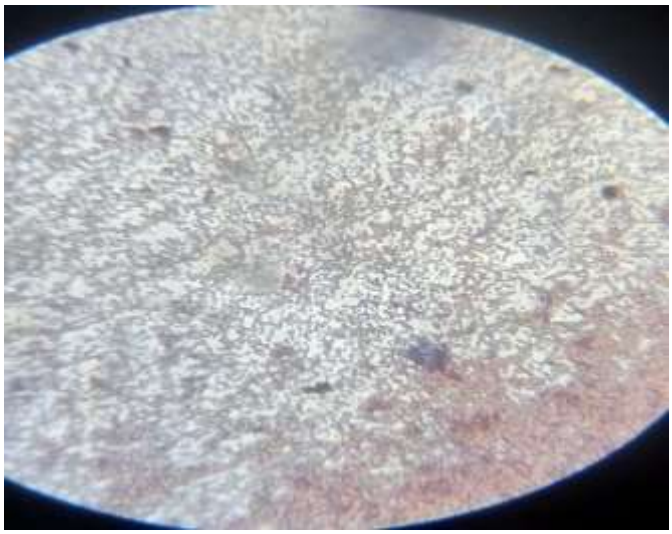

Table 3. Total Bacterial Count in Nutrient Agar

S. No.	Sample	Concentration	Total number of colonies (CFU/ml)	
			24 hours	48 hours
1	Brinjal	10-2	Too numerous	Too numerous
2	Brinjal	10-3	1.92×10^5	1.52×10^5
3	Brinjal	10-5	7.4×10^8	7.3×10^8
4	Potato	10-2	Too numerous	Too numerous
5	Potato	10-3	2.3×10^5	1.3×10^5
6	Potato	10-5	5.7×10^8	9×10^8
7	Carrot	10-2	Too numerous	Too numerous
8	Carrot	10-3	1.09×10^5	5.3×10^5
9	Carrot	10-5	1.2×10^8	1.9×10^8

10	Pumpkin	10-2	Too numerous	Too numerous
11	Pumpkin	10-3	1.76×10^5	1.02×10^5
12	Pumpkin	10-5	3.3×10^8	1.5×10^8
13	Chayote	10-2	Too numerous	Too numerous
14	Chayote	10-3	7.4×10^5	3.5×10^5
15	Chayote	10-5	2.8×10^8	2.5×10^8

Graph 1: Bacterial count of Bacteria in Nutrient agar in both 24 and 48 hours

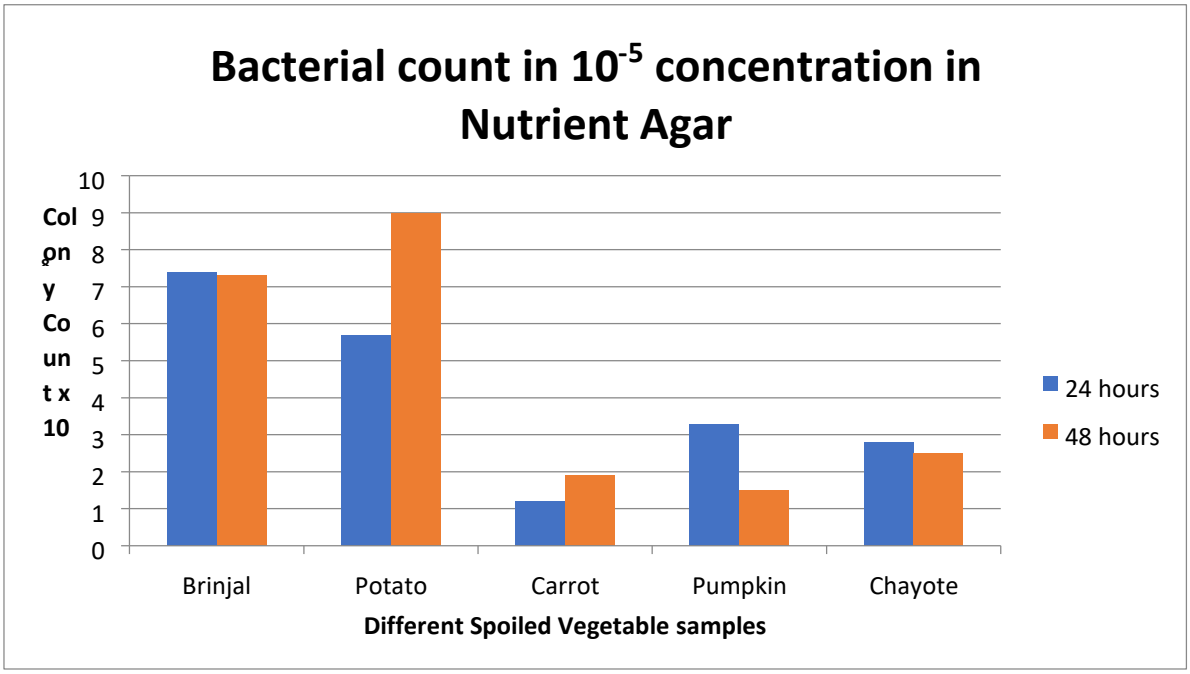

Table 4. Antibacterial activity of Turmeric extract

BACTERIAL CHARACTERISTICS: Gram negative short rods

PREDICTED BACTERIA: *E.coli*

ZONE OF INHIBITION (in mms)

Concentration (%)	Replication 1	Replication 2	Replication 3	Replication 4	Mean
Control (Distilled water)	0	0	0	0	0
Standard (Cephalaxin)	15	18	20	16	17.3
0.625	10	11	11	11	10.75
1.25	11	12	11	12	11.5
5	12	13	12	13	12.5
10	13	13	12	13	12.75
40	14	14	13	14	13.75

Table 5. Antibacterial Activity of *Pongamia* leaf extract

BACTERIAL CHARACTERISTICS: Gram negative short rods

PREDICTED BACTERIA: *E.coli*

ZONE OF INHIBITION (in mms)

Concentration (%)	Replication 1	Replication 2	Replication 3	Replication 4	Mean
Control (Distilled water)	0	0	0	0	0
Standard (Cephalaxin)	19	18	20	17	18.5
0.625	11	9	10	11	10.25
1.25	11	10	11	12	11
5	12	11	13	13	12.25
10	12	12	13	14	12.75
40	13	14	14	15	14

FIG-6: ANTIBACTERIAL ACTIVITY OF BETEL LEAF AGAINST *E.COLI*

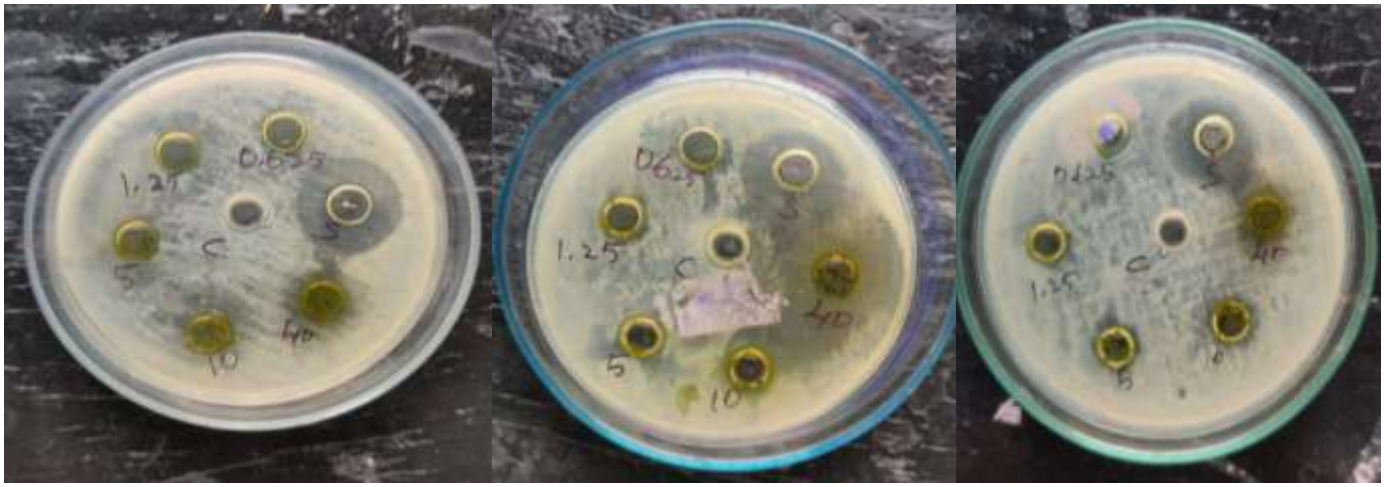

FIG-7: ANTIBACTERIAL ACTIVITY OF *PONGAMIA* AGAINST *E.COLI*

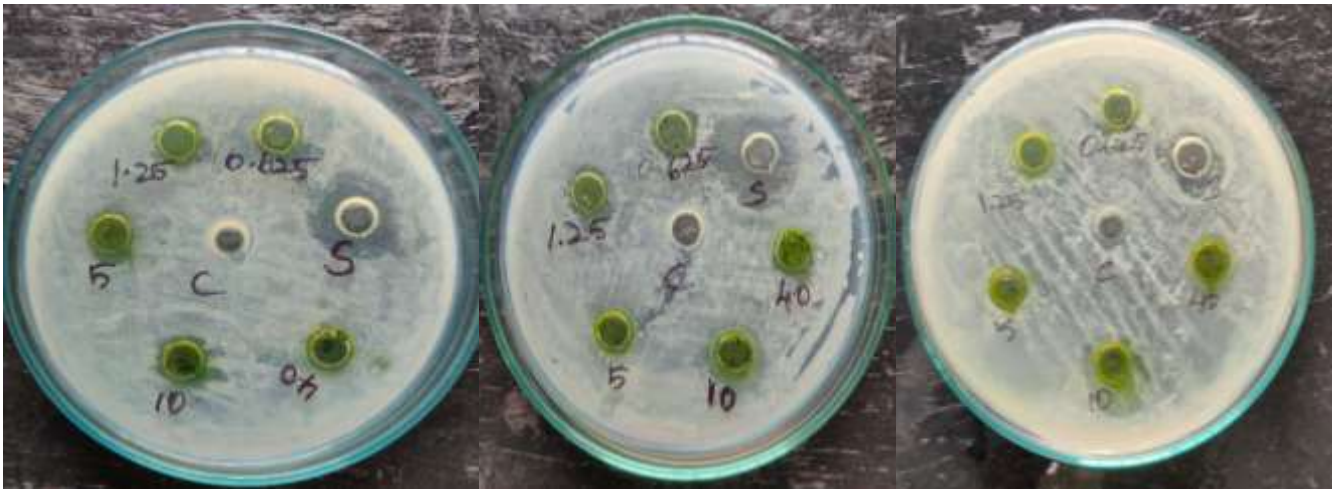

FIG-8: PHYTOCHEMICAL ANALYSIS OF TURMERIC EXTRACT

Pongamia extract

Betal extract

Table 7: Phytochemical analysis of three plant extracts

Phytochemicals	**Turmeric extract**	***Pongamia leaf extract***	**Betel leaf extract**
Alkaloids	+	+	-
Steroids	-	-	-
Tannins	-	-	-
Phlobotannins	-	-	-
Saponins	+	+	+

Flavonoids	-	-	-
Terpenoids	+	-	+
Cardiac glycosides	+	+	-
Phenolic compounds	+	+	+
Aromatic acids	-	-	+
Xanthoproteins	+	-	-

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