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Exploring the efficacy of aqueous extracts of *Malus domestica* var Anna, *Prunus persica, Ricinus communis* and *Carica papaya* against pathogenic bacterial strains

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Abstract

Medicinal plants have antibacterial, antifungal and antiviral activities. More or less all plants have medicinal properties. In this research article, we have selected four economically important plants (three fruit plants and an economically important plant), *Malus domestica* Borkh., *Prunus persica* L., *Ricinus communis* L., and *Carica papaya* L. found in several areas of Indian state Uttarakhand. Using the methanolic extract of leaves, we have screened those four plants against four human pathogenic bacteria, *Escherichia coli*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Micrococcus luteus*. For our experiment we have screened the methanolic leaf extracts of four plants against the above-mentioned bacteria. Statistical analysis was also performed for validation. Result revealed the said bacteria have potential antibacterial activities. So, these leaves can be used for clinical trial. These plants can also be used for making herbal medicines.

1. Introduction

As per World Health Organization (WHO) very less number of modern medicines are developed from medicinal plants. Local people get knowledge of the medicinal properties of plants through their need, observation and the knowledge acquired from ancestors as well as books like spiritual manuscripts. Medicinal plants play important role in killing human pathogens (**Sunilsonson *et al.*, 2016**). *Malus domestica* var. Anna is a famous fruit with several therapeutic phytoconstituents (**Khalifa et al., 2017**). *Malus domestica Borkh.* has several phytoconstituents like; Cinnamic acids, Chlorogenic acid, Caffeic acid, Ferulic acid, P-coumaric acid, Caffeoylquinic acid, P-coumarylquinic acid, Catechin, Epicatechin, Proantho Cyanidins, Flavonols, Quercetin and quercetin Cinnamic glycosides, **(Wolfe, and Liu, R.H., 2003; Eberhardt *et al.*, 2000; Podsedek *et al.,* 2000; Wolfe *et al.*, 2003; He and Liu, 2008; Kumar and Chauhan, 2010; Boyer *et al.*, 2004).** *Prunus persica* is a very famous fruit. It is commonly known as "Peach" in the world wide. This plant is very important for human nutrition **(Kant et al., 2018).** *Prunus persica* L. consists of more than 17 types of phenolics including chlorogenic acid, neochlorogenic acid, caffeoylquinic acid, 3-O-feruloylquinic acid, catechin, procyanidin B1, procyanidin B2, procyanidin dimer, procyanidin C1, procyanidin trimer isomer 1, procyanidin trimer isomer 2 etc. **(Zhang *et al.*, 2019**). Ricinus communis is an economically important oil producing plant (Bolazi et al., 2012). *Ricinus communis* L. plant has Aldehydes (C26 and C28), Alkanes (C26-C29), α-Amyrin , β-Amyrin, N Butylmorpholine, Chlorogenic acid, Camphor, 1,8-Cineole, Citric acid, β- Caryophyllene, Decanamine, N-Demethylricinine , Di-butylphthalate, 2,5-Dihydroxybenzoic acid (Gentisic acid), β-Eleosteric acid, Ellagic acid, (-)-Epicathechin, Fumaric acid, Gallic acid, Hexacosane-1,3-diol, 3-Hexen-1-ylacetate, Kaempferol, Kaempferol 3-O-β-D-glucopyranoside, Kaempferol 3-O-β-D-xylopyranoside, Kaempferol 3-O-β-rutinoside, Linoleic acid, Linolenic acid, Lupeol, Myristic acid, Malic acid, Methyl gallate, Neochlorogenic acid, 4- Octadecylmorpholine, Oleic acid, Palmitic acid, Palmitoleic acid, α-Pinene, Primary alcohols, Quercetin, Hyperoside, Quercetin 3-O-β-Rutinoside,Quercetin-3-O-β-D-glucopyranoside, Quercetin 3-O-β-D-xylopyranoside, Ricinine, β-Sitosterol, Stigmasterol, Stearic acid, Tartaric acid, Tannins **(Singh and Gitanjali, 2015)**. *Carica papaya* L. plant is generally well known for its medicinal properties and potent human health benefits (Madhban 2021). *Carica papaya* L., leaves have phenolic acids, as well as trace amounts of chlorogenic acid, compared to flavonoids and coumarin compounds **(Khuzhaev and Aripova, 2000)**.

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Escherichia coli is a facultative an aerobic, rod-shaped gram-negative bacterium. It is commonly found in lower intestine of warm-blooded animals. It is commonly available in laboratory also. *E. coli* produces Shiga toxin. This bacterium is responsible for gastroenteritis in adult and diarrhoea in infants (Panchagam, 2015). *Streptococcus pneumoniae* is a gram-positive spherical bacterium and the leading bacteria for bacteraemia, meningitis, community acquired pneumonia, Invasive pneumococcal disease (**Loughran et al., 2019**). *Staphylococcus aureus* bacteria is responsible for food poisoning and health harm (**Myles and Datta et al., 2012**). *Staphylococcal* infection mostly present in skin and soft tissues (**Myles and Datta et al., 2012**). *Micrococcus luteus* is a gram-positive coccus. It is normally present in human skin (**Kloos et al., 1974**).

This study focused on identifying the medicinal importance of four economically important plants of Uttarakhand i.e., *Malus domestica* Borkh*., Prunus persica* L., *Ricinus communis* L., and *Carica papaya* L. In this research article, we have identified the antimicrobial role of four above mentioned plants. Our study revealed that, those four plants have potentiality to kill some selected microbes.

2. Materials and methods

   The detailed process of identification of antimicrobial properties of four said plants are given below (Fig 1):

- **Subculture of bacteria-**

➢ Bacterial strains were sub cultured in nutrient broth media.

➢ The nutrient broth was then segregated in twelve test tubes under sterile condition inside the laminar airflow.

➢ After that the test tubes were kept for cooling and bacterial strains were inoculated. Three replicates were maintained.

➢ The test tubes were then kept for 48 hrs in bacterial incubator at 37°C.

- **Antibacterial assay** (**Standard method described by Dhar *et al*., 2016**)

➢ 38 grams of Muller Hilton Agar (MHA) was measured in electronic balance machine and then dissolved 1000 ml of Millipore water. The solution was shaken well and autoclaved at 125°C with 15 psi for 15 minutes.

- 10 ml of each solution was poured into each petri plate under laminar airflow. The media was kept for solidification and then streaking of bacterial strains were performed. Three replicates were maintained for every cases.
- The petri plates were sealed with thin parafilm and kept in bacterial incubator (Bacteriological Shaking incubator – SISCO INDIA PVT LTD.) for growth at 37℃.
- Three wells were developed in each petri plate. In one well 50 µl of 125mg/ml tetracycline antibiotic solution was poured. Antibiotic solution was kept as positive control. In another well 50 µl methanol was poured. In the third well 50 µl of 50mg/ml of leaf extract were poured.
- Total twelve petri plates were prepared by pouring four leaf extracts with three replicates. These experiments were performed for two times by maintain proper biosafety.

- **Measurement of zone of inhibition**

The petri plates were then observed for getting the zone of inhibition. Antibacterial potential was evaluated by measuring the diameters of zone of inhibition in milli meters (mm) with the help of a standard measuring scale.

- **Statistical analysis**

Mean and standard deviation were calculated for each experiment. One way ANOVA was used to estimate the effect of leaf extracts obtained from different plants on inhibition zone of bacterial strains. Least significant difference was calculated for each bacterial strain. Paired sample t test was performed to test the significant difference between leaf extract and antibiotic control.

- **Activity index measurement**

Activity index measurement was performed using the following formula –

$$\textbf{Activity index (AI)} = \frac{\textbf{Zone of inhibition of leaf extract}}{\textbf{Zone of inhibition of Tetracycline}}$$

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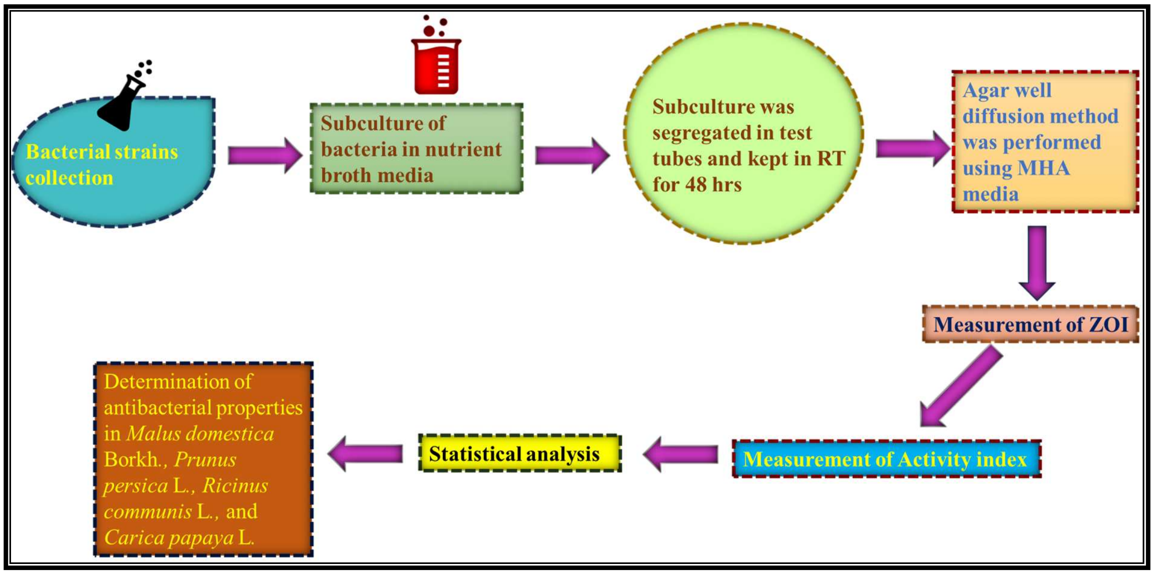

Fig 1: A schematic representation of the total antimicrobial properties identification processes of four economically important plants; *Malus domestica* Borkh., *Prunus persica* L., Carica papaya L., and *Ricinus communis* L.

3. Results

- **Results of the experiments performed to determine the antibacterial efficacy of four said plants:**

The inhibitory effect of leaf extracts from *Malus domestica*, *Prunus persica*, *Carica papaya* and *Ricinus communis* was tested against Bacterial strains of *Escherichia coli* (MTCC 40), *Streptococcus pneumoniae* (MTCC 655), *Staphylococcus aureus* (MTCC 96), and *Micrococcus luteus* (MTTC 106) at a concentration of 50 mg ml−1, resulted in different extents of inhibition (Table 1 and 2, Graph 1, Fig 2). Inhibition was checked using leaf extracts. *Ricinus communis* has no inhibitory effect on *Escherichia coli*. *Carica papaya* has best antagonistic effect on bacterial strains. *Micrococcus luteus* was found as most sensitive microorganism to the extracts examined in the study. Measurement of activity index was performed for four said plants (Table 3). *Malus domestica* activity index against *Escherichia coli* was 0.62, against *Streptococcus pneumoniae* was 0.86, against *Staphylococcus aureus* was 1.56 and *Micrococcus luteus* was 4.07. In *Prunus persica* activity index against *Escherichia coli* was 0.94, against *Streptococcus pneumoniae* was 0.50, against *Staphylococcus aureus* was 1.73 and *Micrococcus luteus* was 2.30. *Carica papaya* activity index against *Escherichia coli* was 0.49, against *Streptococcus pneumoniae* was 0.56, against *Staphylococcus aureus* was 2.43 and *Micrococcus luteus* was 5.89. *Ricinus communis* activity

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index against *Streptococcus pneumoniae* was 0.86, against *Staphylococcus aureus* was 1.56 and *Micrococcus luteus* was 4.07. Highest activity index was found in *Carica papaya* against *Micrococcus luteus* (Table 3, Fig 2, 3. 4,5, 6). One way ANOVA was performed to analyse significance of the collected data. *Carica papaya* showed highest significance among the variables (Table1,2,3).

Table 1: Determination of antibacterial efficacy of *Malus domestica*, *Carica papaya*, *Prunus persica*, *Ricinus communis* plants

Sn.	Plant name	Bacterial strains used	Presence of zone of inhibition
1	*Malus domestica* var Anna	*Escherichia coli*	Yes
2	*Malus domestica* var Anna	*Streptococcus pneumoniae*	Yes
3	*Malus domestica* var Anna	*Staphylococcus aureus*	Yes
4	*Malus domestica* var Anna	*Micrococcus luteus*	Yes
5	*Prunus persica*	*Escherichia coli*	Yes
6	*Prunus persica*	*Streptococcus pneumoniae*	Yes
7	*Prunus persica*	*Staphylococcus aureus*	Yes
9	*Ricinus communis*	*Escherichia coli*	No
10	*Ricinus communis*	*Streptococcus pneumoniae*	Yes
11	*Ricinus communis*	*Staphylococcus aureus*	Yes
12	*Ricinus communis*	*Micrococcus luteus*	Yes
13	*Carica papaya*	*Escherichia coli*	Yes
14	*Carica papaya*	*Streptococcus pneumoniae*	Yes
15	*Carica papaya*	*Staphylococcus aureus*	Yes
16	*Carica papaya*	*Micrococcus luteus*	Yes

Table 2: Data of the measurement of the diameter of the zone of inhibitions produced by *Malus domestica*, *Carica papaya*, *Prunus persica* and *Ricinus communis*

Bacterial strains	Plant name	Mean value of zone of inhibition by leaf extract (mm)	Mean of inhibition of positive control (mm)	T value	P value

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Escherichia coli	*Malus domestica* var Anna	3.3±0.47	5.3±0.47	6.93	0.02
	Prunus persica	5±0.81	5.3±0.47	-1	0.42
	Carica papaya	2.6±0.47	5.3±0.47	-8	0.01
LSD (α=0.05)		1.82			
Streptococcus pneumoniae	*Malus domestica* var Anna	4.6±0.94	5.3±0.47	-0.75	0.5
	Prunus persica	2.7±0.47	5.3±0.47	-4	0.05
	Ricinus communis	3.6±0.47	5.3±0.47	0.18	0.87
	Carica papaya	3±0.81	5.3±0.47	-3.5	0.07
LSD (α=0.05)		2.31			
Staphylococcus aureus	*Malus domestica* var Anna	3.6±0.47	2.3±0.47	2	0.18
	Prunus persica	4±0.47	2.3±0.47	2	0.18
	Ricinus communis	3.6±0.47	2.3±0.47	4	0.05
	Carica papaya	5.3±0.47	2.3±0.47	19.5	0.003
LSD (α=0.05)		1.54			
Micrococcus luteus	*Malus domestica* var Anna	5.3±0.47	1.3±0.47	6.92	0.02
	Prunus persica	3±0.47	1.3±0.47	2	0.18
	Ricinus communis	2.3±0.47	1.3±0.47	3.4	0.07
	Carica papaya	7.66±0.47	1.3±0.47	19	0.003
LSD (α=0.05)		1.54			

Table 3: Determination of Activity Index for *Malus domestica, Prunus persica, Ricinus communis* and *Carica papaya* leaf extracts

Bacterial strains	Plant name	Activity Index
Escherichia coli	*Malus domestica* var Anna	0.62
Streptococcus pneumoniae	*Malus domestica* var Anna	0.86
Staphylococcus aureus	*Malus domestica* var Anna	1.56

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Micrococcus luteus	*Malus domestica* var Anna	4.07
Escherichia coli	*Prunus persica*	0.94
Streptococcus pneumoniae	*Prunus persica*	0.50
Staphylococcus aureus	*Prunus persica*	1.73
Micrococcus luteus	*Prunus persica*	2.30
Streptococcus pneumoniae	*Ricinus communis*	0.67
Staphylococcus aureus	*Ricinus communis*	1.56
Micrococcus luteus	*Ricinus communis*	1.76
Escherichia coli	*Carica papaya*	0.49
Streptococcus pneumoniae	*Carica papaya*	0.56
Staphylococcus aureus	*Carica papaya*	2.43
Micrococcus luteus	*Carica papaya*	5.89

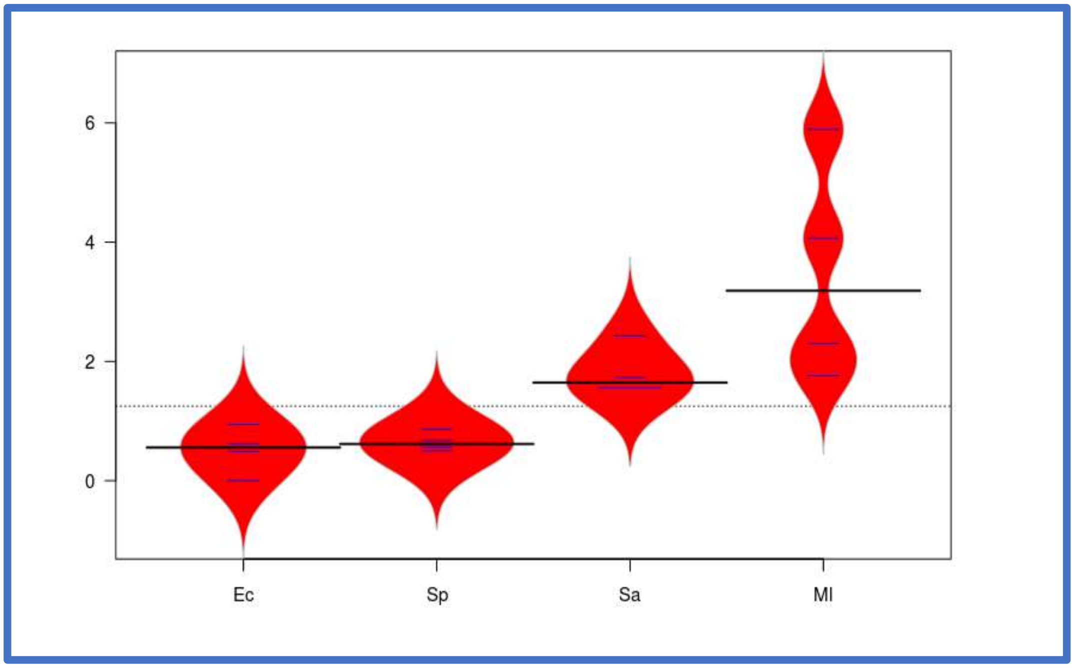

Fig 2: Bean plot of data distribution was performed using bean plot package R (https://cran.r-project.org/package=beanplot) to analyse the activity index of four said plants against four said pathogenic bacterial strains

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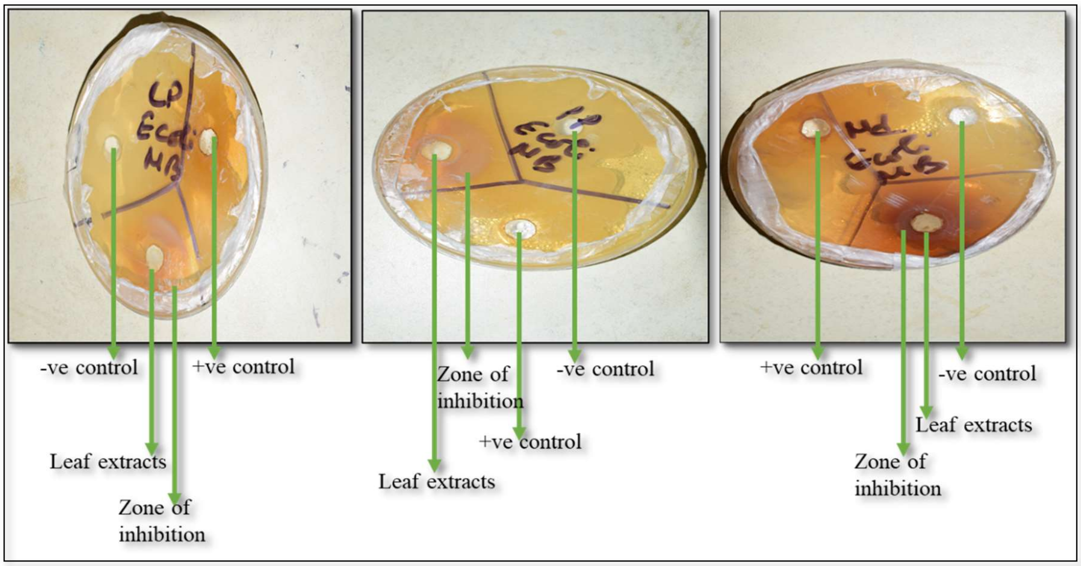

Fig 3. Petri plates with significant zone of inhibitions against *E. coli* bacteria: from left to right Petri plates contain *Carica papaya* leaf extract, *Prunus persica* leaf extract and *Malus domestica* leaf extract. Here positive control contains Tetracycline antibiotic and negative control contains autoclaved millipore water

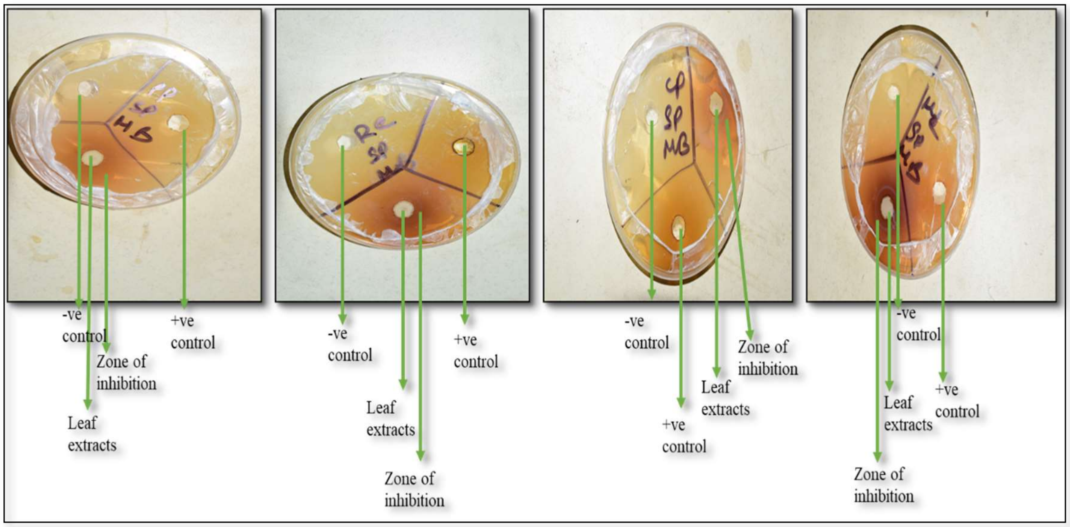

Fig 4. Petri plates with significant zone of inhibitions against *Streptococcus pneumoniae* bacteria: from left to right Petri plates contain, *Prunus persica* leaf extract, *Ricinus communis* leaf extract, *Carica papaya* leaf extract and *Malus domestica* leaf extract. Here positive control contains Tetracycline antibiotic and negative control contains autoclaved Millipore water

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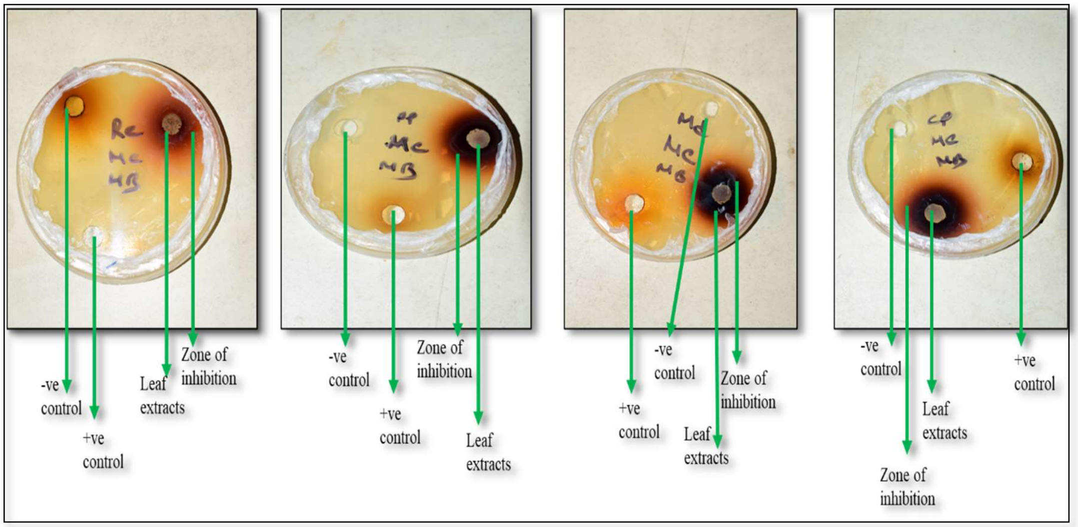


Fig 5: Petri plates with significant zone of inhibitions against *Micrococcus luteus* bacteria: from left to right Petri plates contain, *Ricinus communis* leaf extract, *Prunus persica* leaf extract, *Malus domestica* leaf extract, and *Carica papaya* leaf extract. Here positive control contains Tetracycline antibiotic and negative control contains autoclaved Millipore water

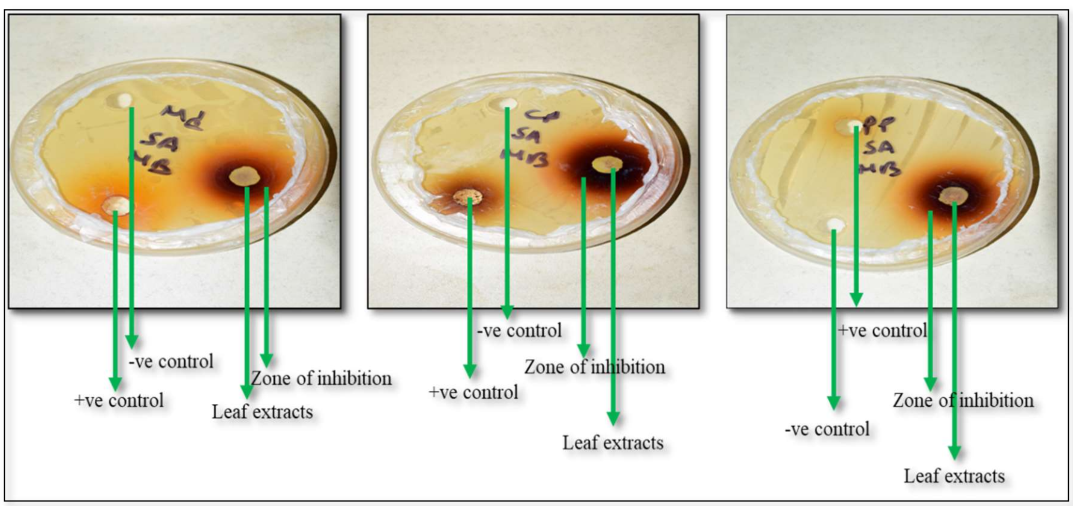
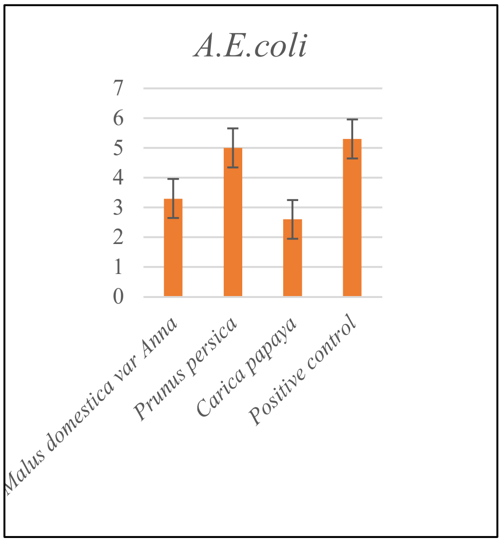
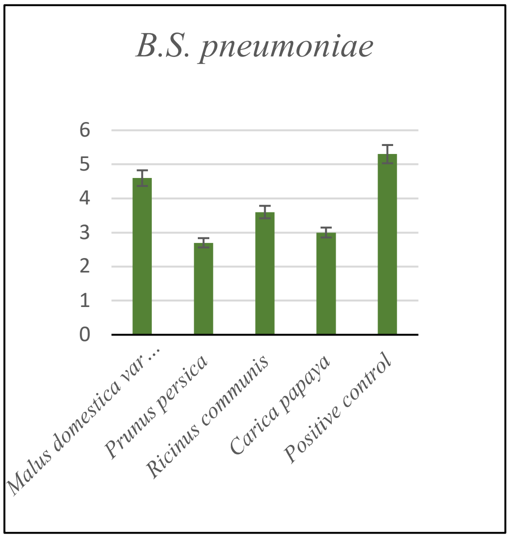


Fig 6: Petri plates with significant zone of inhibitions against *Staphylococcus aureus* bacteria: from left to right Petri plates contain, *Malus domestica* leaf extract, *Carica papaya* leaf extract and *Prunus persica* leaf extract. Here positive control contains Tetracycline antibiotic and negative control contains autoclaved Millipore water


A.E.coli
7
6
5
4
3
2
1
0
Malus domestica var Anna
Prunus persica
Carica papaya
Positive control


B.S. pneumoniae
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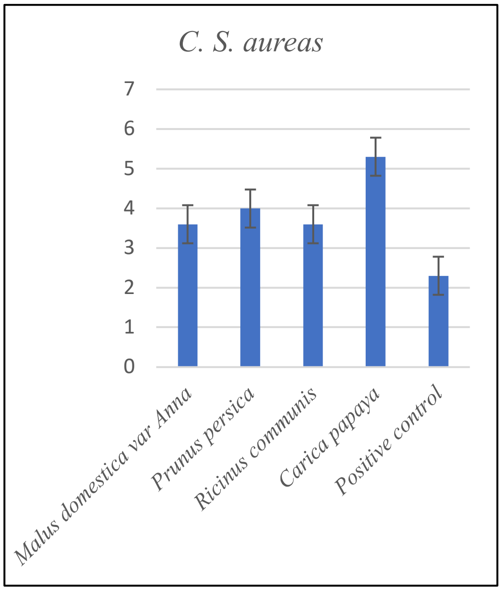
Malus domestica var ...
Prunus persica
Ricinus communis
Carica papaya
Positive control


C. S. aureas
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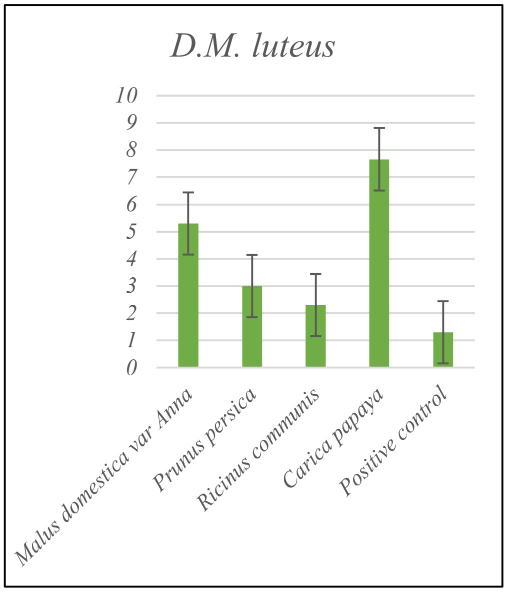
Malus domestica var Anna
Prunus persica
Ricinus communis
Carica papaya
Positive control


D.M. luteus
10
9
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1
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Malus domestica var Anna
Prunus persica
Ricinus communis
Carica papaya
Positive control

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Graph 1: Comparative analysis of the measured zone of inhibition diameter by four above mentioned plants' leaf extract and positive control A. against *E. coli* B. *Streptococcus pneumoniae*, C. *Staphylococcus aureus* and D. *Micrococcus luteus*

4. Discussion

Antimicrobial activity of above mentioned four plants was tested against four pathogenic bacterial strains named, *Escherichia coli*, *Micrococcus luteus, Streptococcus pneumoniae* and *Staphylococcus aureus* leaf extracts were screened against four pathogenic bacterial strains using agar well diffusion method **(Bhat *et al*., 2016)**. Our study is to find out the activity index i.e., higher antagonistic activity of leaf extracts than commercially available antibiotics against pathogenic bacterial strains. For that, Activity index was calculated where the zone of inhibition is higher than antibiotics using previous methods described by **Siddique *et al*., 2017**. Previous studies (**Siddique *et al*., 2017**) reported that, *Malus domestica* has moderate antimicrobial activities against *Escherichia coli* and *Staphylococcus aureus. Malus domestica* also has antimicrobial activities against a pneumonia strain *Klebsiella pneumoniae* (**Siddique and Liya 2018**). It was reported that, *Prunus persica* plant has moderate effect against *Escherichia coli* and *Staphylococcus aureus* and low activity against *Klebsiella pneumoniae* **(Bayih and Abdulhakim 2018)**. Previous studies also revealed that, *Ricinus communis* has bioactive compounds which has antimicrobial potentialities against *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* **(**Sebola ***et al*., 2020)**. *Carica papaya* leaves and seeds has great antimicrobial activities against *Streptococcus pneumoniae*, *Escherichia coli* and *Staphylococcus aureus* **(Ayanfemi and Bukola, 2015)**. It has moderate activity against *Micrococcus luteus* **(Baskaran *et al*., 2012)**. After screening it was identified that three plants, i.e., *Malus domestica, Prunus persica* and *Carica papaya* leaf extracts has both *Escherichia coli* and *Micrococcus luteus* bacteria suppressing ability. All four plants have antagonistic effect against both *Streptococcus pneumoniae* and *Micrococcus luteus*. After comparing with the radii of zone of inhibition of tetracycline antibiotic and leaf extracts eight cases were found where the zone of inhibition radii was higher in leaf extracts than antibiotic. All four plants' leaf extracts show higher radii of zone of inhibition against *Micrococcus luteus* and *Staphylococcus aureus*. *Micrococcus luteus* is the most susceptible bacteria. *Carica papaya* shows the highest antimicrobial activity against *Micrococcus luteus*. The radii of zone of inhibition of *Carica papaya* leaf extracts against *Micrococcus luteus* is highest and its range was from 7-8 mm ± 0.47 mm.

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Activity index was measured using the standard formula used by **Siddique *et al.*, 2017.** From the recorded activity index *Carica papaya* showed highest activity index. Among four plants, *Carica papaya* has highest activity index against *Micrococcus luteus*, *Staphylococcus aureus*; Malus domestica has highest activity index against *Streptococcus pneumoniae; Prunus persica* has highest antimicrobial activity against *Escherichia coli.*

T test analysis between paired sampled t test (zone of inhibition using leaf extracts and zone of inhibition using tetracycline antibiotic) against four pathogenic strains performed in SPSS revealed significant output in *Malus domestica* against *Escherichia coli* and *Micrococcus luteus* strains. Significant outputs are also explored in *Carica papaya* against *Escherichia coli*, *Staphylococcus aureus* and *Micrococcus luteus* strain. Multiple comparisons on the basis of susceptibility were performed using one way ANOVA of *Malus domestica* Borkh., *Carica papaya* L., *Prunus persica* L., *Ricinus communis* L. in SPSS version 22. Results revealed that *Escherichia coli*, *Staphylococcus aureus* and *Micrococcus luteus* strains are highly susceptible in the leaf extracts of four above mentioned plants. Statistical analysis explored *Micrococcus luteus* is the most susceptible bacterial strain. Multiple comparisons using the parameter "zone of inhibition by different leaf extracts" was performed to differentiate between four leaf extracts.

5. Summary

- From the present study, it can be advised to farmers that, rapid agriculture and organic farming of *Carica papaya* L., *Ricinus communis* L and *Malus domestica* Borkh. can be cultivated in large scale. Oral intake of *Carica papaya* leaf juice is highly recommended. Use of *Ricinus communis* leaves are recommended to heal pains. Local people can propagate these plants more in their fields and domestic areas to get health benefits naturally.

6. Conclusion

From antibacterial assays it can be concluded that herbal drugs against *Staphylococcus aureus* and *Micrococcus luteus* can be possible. *Carica papaya* has the highest bacteria suppressing activity among these four plants. Clinical trial of oral application of *Carica papaya* leaf extracts can be possible for patients who are suffering from *Micrococcus luteus* and *Staphylococcus aureus* infections.

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