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Antimicrobial activity of different rhizomes on Streptococcus pneumoniae and Proteus vulgaris and preservative efficacy of rhizomes Solanum tuberosum, Ipomoea batatas, Zingiber officinale, Curcuma longa ad Colocasia esculenta on commercial Yoghurt

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ABSTRACT

Multidrug resistance in microbes is one of the major concern now a days. Development of novel drugs formulated from natural compounds can help in preventing the development of resistance and in this work we have used five different rhizomes to study their anti microbial effect on four different bacterial strains like Pseudomonas aeuroginosa, Proteus vulgaris, Citrobacter freundii. and Steptococcus pneumoniae isolated from biological samples are used. Ipomoea batatas, Zingiber officinale, Curcuma longa and Colocasia esculenta proven to be effective against S.pneumoniae with recorded IC50 of around 5mg/50µL and 8mg/80µL. Where as Ipomoea batatas, Curcuma longa and Colocasia esculenta proven to be effective against P.vulgaris with high IC50 value compared to S.pneumoniae. All the five rhizomes Solanum tuberosum, Ipomoea batatas, Zingiber officinale, Curcuma longa ad Colocasia esculenta are effective preservatives in preserving the commercial yogurt due to presence of secondary metabolites like Alkaloids, Phenols, Tannins and flavonoids.

KEYWORDS: Solanum tuberosum, Ipomoea batatas, Zingiber officinale, Curcuma longa and Colocasia esculenta, Pseudomonas aeuroginosa, Proteus vulgaris, Citrobacter freundii. and Steptococcus pneumoniae, Anti microbial activity, Preservative efficacy

INTRODUCTION

Solanum tuberosum contains endotoxin solanine that is poisonous when the concentration exceeds more than 200mg/kg. Studies of Liu et al., (2020) proven that bacterial strains like P-NA2-14 (Bacillus megaterium NBRC 15308T) can inhibit the solanine production and endophytic bacterial growth in tubers there by improving the storage period of crop. *Ipomoea batatas* commonly known as sweet potato is an important phytochemical source of energy and animal nutrition. From the studies of Pochapski et al., (2011), *Ipomoea batatas* leaf extract cannot inhibit the growth of bacterial strains like *Streptococcus mutans, S. mitis, Staphylococcus aureus,* and *Candida albicans.*

Ginger (Zingiber officinale) is commonly used to treat some of the common diseases and preferred as herbal medicine from olden era. From the studies of Ahmed et al., (2022) *Zingiber officinale* is an effective antibacterial agent against gram positive bacteria like *Streptococcus mutans* and selective oral microbes. *Curcuma longa* is known for its antibacterial activity against various gram positive and gram negative bacteria due to presence of curcuminoids (Hussain et al., (2022)). Curcumin loaded nanoparticles are proven to be effective against *S. aureus* isolated from periprosthetic joint infection samples (Hussain et al., (2022)).

Colocasia esculenta Linn. Leaves and tubers are well known for their antimicrobial properties due to the secondary metabolite content they contain (Mohini B et al., (2024)) and hydroalcoholic leaf extract of *Colocasia esculenta* proven to toxic against bacteria like *S. Aureus, P. Aeruginosa* and others (Mohini B et

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al., (2024)). In current study we have used rhizomes like *Solanum tuberosum*, *Ipomoea batatas, Zingiber officinale, Curcuma longa* and *Colocasia esculenta* to study the antibacterial activity against *Pseudomonas aeuroginosa, Proteus vulgaris, Citrobacter freundii.* and *Steptococcus pneumoniae* isolated from various biological samples.

METHODOLOGY:

Isolation of *Pseudomonas aeuroginosa, Proteus vulgaris, Citrobacter freundii.* and *Steptococcus pneumoniae* from Biological samples:

Pseudomonas aeuroginosa is isolated from the sewage water where as *Proteus vulgaris, Citrobacter freundii.* and *Steptococcus pneumoniae* were isolated from toilet flush water (*Proteus vulgaris)*, roots of Murray koeingii (*Citrobacter freundii.)* and nasopharynx sample (*Steptococcus pneumoniae)* followed by isolation using serial dilution and spread plating methods.

Phytochemical analysis of *Solanum tuberosum*, *Ipomoea batatas, Zingiber officinale, Curcuma longa* and *Colocasia esculenta:*

Phytochemical tests were performed using standard qualitative procedures to detect the presence of various bioactive constituents, including carbohydrates, phenols, tannins, alkaloids, flavonoids, proteins, and ketones (Beeram. E et al.,(2023)).

2.1 Tests for Carbohydrates

Fehling's Test

To 1 mL of extract, a few drops each of Fehling's A and B solutions were added and heated. A reddish-brown precipitate indicates the presence of reducing sugars.

Benedict's Test

1 mL of extract is added with Benedict's reagent and heated under flame for few minutes. A red or brick-red precipitate confirms the presence of reducing sugars.

Barfoed's Test

To 1 mL of extract, Barfoed's reagent was added and boiled for few minutes in a water bath. Formation of a brickish-brown precipitate within 1–2 minutes indicates presence of monosaccharides.

Molisch Test

To 1 mL of extract, a few drops of alpha-naphthol solution is added followed by Conc.H2SO4. A violet or purple ring at the interface confirms the carbohydrate presence.

2.2 Rothera's Nitroprusside Test (For Ketone Bodies)

To 1 mL of extract, ammonium sulphate was added till saturation. Then 2–3 drops of freshly prepared sodium nitroprusside solution (0.5%) is added , followed by addition of 2 mL of ammonia. Purple color formation indicates the presence of ketone compounds.

2.3 Test for Phenols and Tannins

Ferric Chloride Test

To 1 mL of extract, 2–3 drops of 10% $FeCl_3$ is added and change in colour from reddish brown to green or blue-black indicates presence of phenols or tannins.

2.4 Tests for Alkaloids

Wagner's Test

To 1 mL of extract, a few drops of Wagner's reagent (iodine in potassium iodide) is added and formation of yellow or brown precipitate indicates alkaloids presence in the phytochemical.

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2.5 Test for Flavonoids

Alkali Reagent Test

To 1 mL of extract, 2–3 drops of sodium hydroxide were added. A yellow colour appearance that turns colourless up on addition of dilute HCl, indicates the presence of flavonoids.

2.6 Ninhydrin Test

To 1 mL of extract, a few drops of ninhydrin reagent were added, and the mixture was boiled in a water bath. Formation of a blue or purple colour confirms the presence of amino acids or proteins.

2.7 Test for Ketones (Resorcinol Test)

To 1 mL of extract, a few crystals of resorcinol and equal volumes of concentrated HCl is added and the mixture is allowed to heat over a spirit lamp. Apperance of pink color indicates the presence of ketones.

IMVIC Tests:

Indole test, Methyl red test, Voges - Prausker test and Citrate utilization test are performed according to standard procedures with the bacterial strains *Pseudomonas aeuroginosa, Proteus vulgaris, Citrobacter freundii. and Steptococcus pneumoniae.*

Anti microbial susceptibility test:

Anti microbial activity of phytochemicals *Solanum tuberosum*, *Ipomoea batatas, Zingiber officinale, Curcuma longa* and *Colocasia esculenta* is tested against the bacteria *Pseudomonas aeuroginosa, Proteus vulgaris, Citrobacter freundii.* and *Steptococcus pneumoniae* using agar well diffusion technique or method.

Preservation of Yoghurt using Phytochemicals *Solanum tuberosum*, *Ipomoea batatas, Zingiber officinale, Curcuma longa* and *Colocasia esculenta:*

10ml of curd sample is transferred aseptically in to four separate beakers in the laminar air flow chamber and 0.1 g of each phytochemical is transferred aseptically in to respective labeled beakers containing curd and kept under aseptic conditions for 24 hrs. On the next day the microbial load present in the curd is assessed by using spread plating method and counting the number of colonies formed on the agar plate on the next day.

For confirmation of the bacteria grown on the agar plate after preservation Gram staining protol is followed.

Gram staining:

Gram staining procedure is followed according to Gram, Hans Christian (1884).

Results:

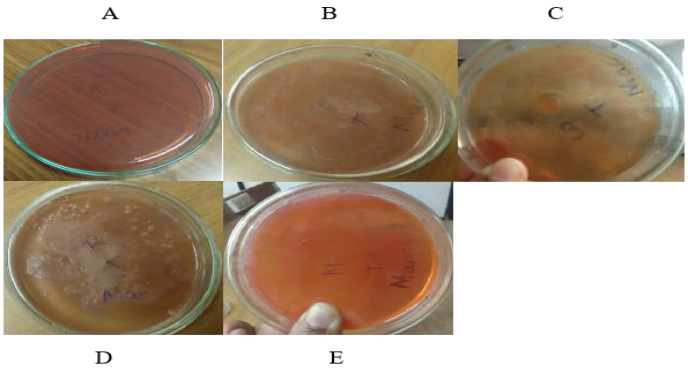

Figure:1 Isolation and Characterization of *Pseudomonas aeuroginosa, Proteus vulgaris, Citrobacter Sps.* and *Steptococcus pneumoniae* using Macconkey agar and Mannitol salt agar. (A) Control, (B) *P.aeuroginosa*, (C) *Citrobacter Sps*. (D) *S.pneumoniae*.

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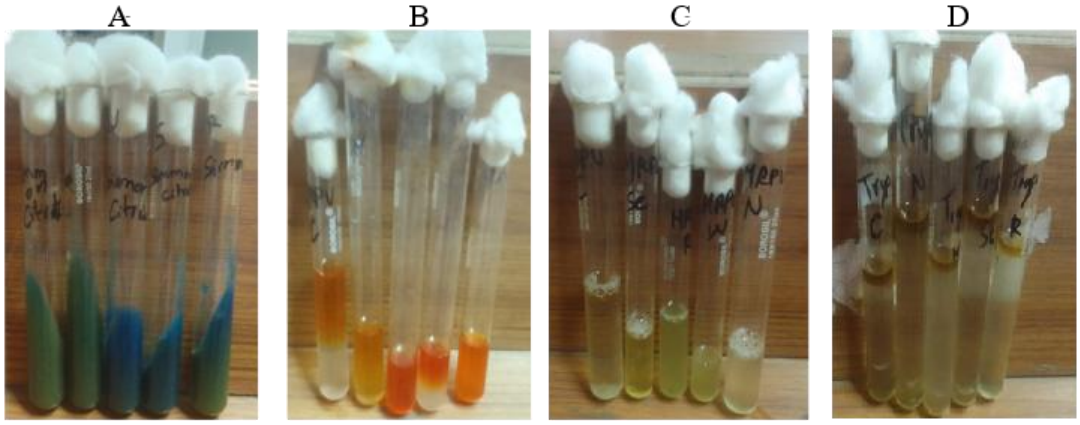

Figure:2 Biochemical characterization of bacteria isolated by Macconkey agar and Mannitol salt agar using IMVIC tests : (A) Citrate test (+ ve : *Proteus*, *Citrobacter Sps.*, *P.aeruginosa*. (B) Methyl red test (+ ve: *Proteus, Citrobacter Sps*.). (C) Voges - Prousker test (+ ve: No strain). (D) Indole test: (+ : No strain).

From the fig.1 &2 the isolated bacteria from sewage water, toilet flush water, nasal sample and root sample of Murray koeingii are identified as *P.aeruginosa, P.vulgaris, S.pneumoniae and Citrobacter freundii.* based on the lactose fermentation in macconkey agar and morphological characteristics and colony consistency observed on Mannitol salt agar plate.

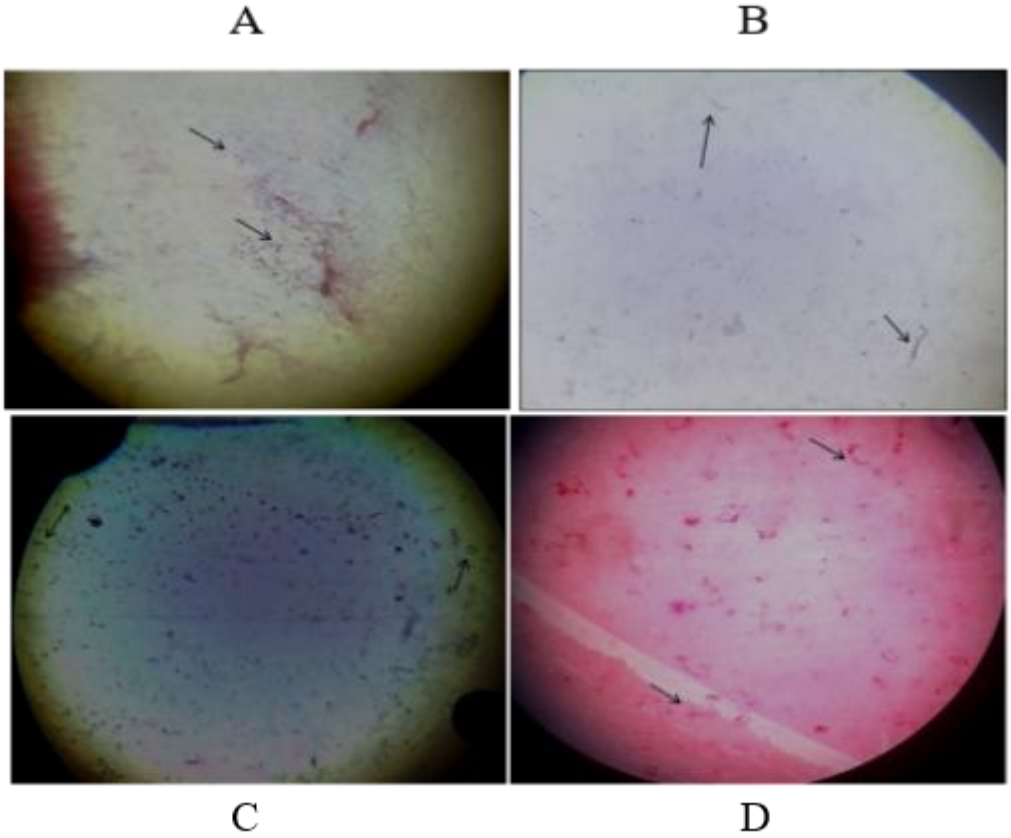

Figure:3 Characterization of bacteria grown on macconkey agar plate and mannitol salt agar plate using Gram staining technique. (A) *S.pneumoniae* (B) *Proteus vulgaris* (C) *P.aeruginosa* (D) *Citrobacter sps*.

From figure 3 the bacteria isolated from the toilet flush water is identified as a gram negative rod *P.vulgaris* with peritrichous flagella forming pink colonies on macconkey agar. Bacteria isolated from sewage water is confirmed as *P.aeuroginosa* a gram negative rod and non lactose fermenter with a single flagellum at polar

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end. Bacteria collected from nasal sample is identified as *S.pneumoniae* characterized as gram positive cocci with negative results for all the IMVIC tests. Murray koeingii root is washed with water and used as source for the bacterial sample and the bacteria present in the sample is confirmed as *Citrobacter freundii* a gram negative rod and is a slow lactose fermenter with negative result for indole test and is usually positive for Citrobacter koseri.

S.No	Contents	*Solanum tuberosum* (Potato)	*Ipomoea batatas* (Sweet Potato)	*Curcuma longa* (Turmeric)	*Zingiber officinale* (Ginger)	*Colocasia esculenta* (Taro)
.	Tests for Carbohydrate Fehling's test	-VE	-VE	-VE	-VE	-VE
.	Benedicts test	-VE	-VE	-VE	-VE	-VE
.	Barfoed test	-VE	+VE	-VE	-VE	-VE
.	Molisch Test	+VE	+VE	+VE	+VE	+VE
.	Test for ketones	-VE	-VE	-VE	-VE	-VE
.	Rothera's or Nitroprusside test	-VE	-VE	-VE	-VE	-VE
	Test for phenols and tannins. 10% Fecl3	+VE	-VE	-VE	-VE	-VE
.	Test for alkaloids	-VE	-VE	-VE	+VE	+VE
.	Test for flavonoids	+VE	+VE	+VE	-VE	-VE

Table: 1 Phytochemical analysis of aqueous extract of *Solanum tuberosum*, *Ipomoea batatas, Zingiber officinale, Curcuma longa* ad *Colocasia esculenta* [+ve = Positive result; - ve = negative result].

From table: 1 the aqueous extract of phytochemical *Solanum tuberosum* contains non reducing polysaccharides, Phenols, tannins and flvanoids and *Ipomoea batatas* possess non reducing disaccharides, polysaccharides and Flavanoids. Aqueous extract of *Curcuma longa* contains polysaccharides and flavanoids where as *Zingiber officinale & Colocasia esculenta* contains polysaccharides and alkaloids.

S.No	Contents	*Solanum tuberosum* (Potato)	*Ipomoea batatas* (Sweet Potato)	*Curcuma longa* (Turmeric)	*Zingiber officinale* (Ginger)	*Colocasia esculenta* (Taro)
1.	Tests for Carbohydrate Fehling's test	-VE	-VE	-VE	-VE	-VE
2.	Benedicts test	-VE	-VE	-VE	-VE	-VE
3.	Barfoed test	-VE	-VE	-VE	-VE	-VE
4.	Molisch test	+VE	+VE	+VE	+VE	+VE
5.	Test for ketones	-VE	-VE	-VE	-VE	-VE
6.	Rothera's or Nitroprusside test	-VE	-VE	-VE	-VE	-VE

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7.	Test for phenols and tannins 10% Fecl3	-VE	-VE	-VE	-VE	-VE
8.	Test for alkaloids	-VE	-VE	-VE	-VE	-VE
9.	Test for flavonoids	+VE	-VE	+VE	+VE	-VE

Table: 2 : Phytochemical analysis tests for *Solanum tuberosum, Ipomoea batatas*, *Zingiber officinale, Curcuma longa* ad *Colocasia esculenta* (Hexane Extract).[+ve = Positive result; -ve = negative result].

Hexane extract of *Solanum tuberosum,Curcuma longa & Zingiber officinale* shown to possess polysaccharides and flavanoids and whereas *Ipomoea batatas & Colocasia esculenta* contains only polysaccharides.

S.No	**Contents**	***Solanum tuberosum* (Potato)**	***Ipomoea batatas* (Sweet Potato)**	***Curcuma longa* (Turmeric)**	***Zingiber officinale* (Ginger)**	***Colocasia esculenta* (Taro)**
1.	Tests for Carbohydrate Fehling's test	-VE	-VE	-VE	-VE	-VE
2.	Benedicts test	-VE	-VE	-VE	-VE	-VE
3.	Barfoed test	-VE	+VE	-VE	-VE	-VE
4.	Molisch test	+VE	+VE	+VE	+VE	+VE
5.	Test for ketones	-VE	-VE	-VE	-VE	-VE
6.	Rothera's or Nitroprusside test	-VE	-VE	-VE	-VE	-VE
	Test for phenols and tannins 10% Fecl3	+VE	-VE	-VE	-VE	-VE
8.	Test for alkaloids	-VE	-VE	+VE	-VE	-VE
9.	Test for flavonoids	-VE	+VE	-VE	+VE	-VE

Table: 3 : Phytochemical analysis of ethanolic plant extracts of *Solanum tuberosum, Ipomoea batatas, Zingiber officinale, Curcuma longa ad Colocasia esculenta* .

Ethanolic tuber extracts of *Solanum tuberosum* possess polysaccharides, Phenols & tannins and *Ipomoea batatas* contains non reducing disaccharides and flavanoids. Where as *Curcuma longa* posess polysaccharides and alkaloids and *Zingiber officinale* shown to contain polysaccharides and flavanoids. *Colocasia esculenta* ethanolic extract shown to possess only polysaccharides.

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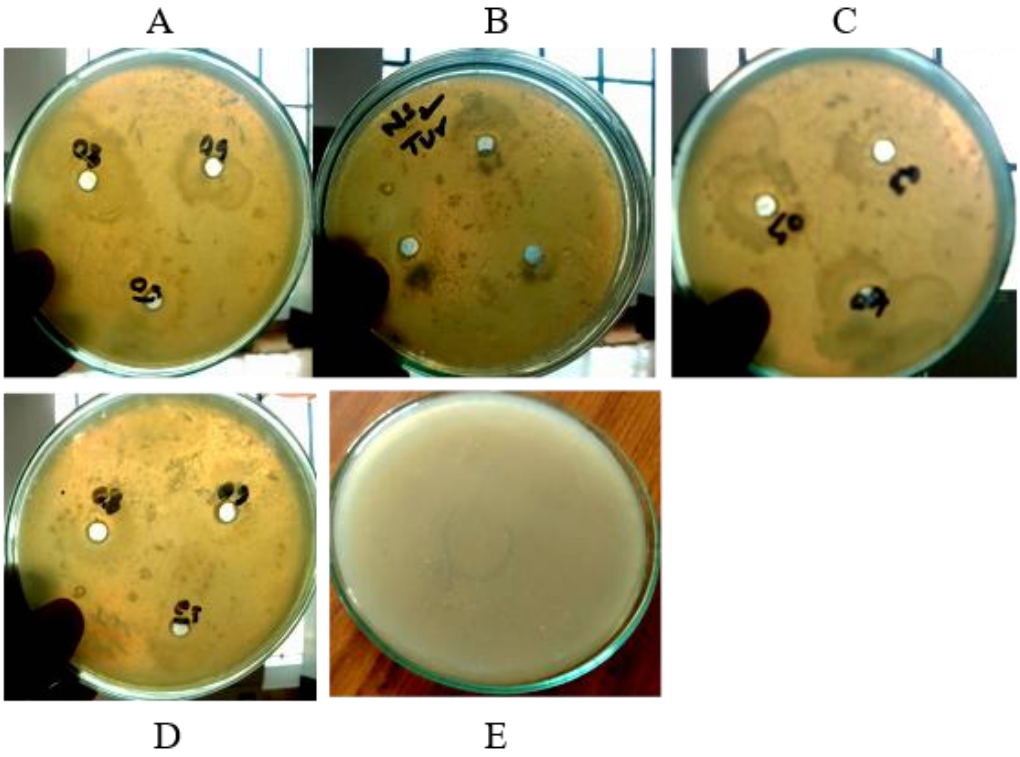

Figure: 4 Antimicrobial activity of (C) *Ipomoea batatas,* (A) *Zingiber officinale*, (B) *Curcuma longa* and (D) *Colocasia esculenta* on *S.pneumoniae*.

Zingiber officinale, Curcuma longa, Ipomoea batatas, and Colocasia esculenta has shown to possess antimicrobial property with zones of inhibition of 0.1 mm (50 µL), 1.0 mm (60 µL) and 1.4mm (80µL) with IC_{50} of about 8mg/80µL (*Colocasia esculenta)* and 1.6mm (50 µL), 1.5 mm (60 µL) and 1.3mm (80µL) and IC_{50} with 5mg/50 µL (*Ipomoea batatas). For C.longa* the recorded diameters of zones of inhibition is found to be 1.3mm (50 µL), 0 mm (60 µL) and 1.3mm (80µL) with IC_{50} value of 5mg/50 µL and for *Zingiber officinale* zones of inhibition formed includes 0mm(50 µL), 0 mm (60 µL) and 1.4 mm (80µL) with IC_{50} value of about 8mg/80µl for the strain S.pneumoniae. In all four phytochemicals *Ipomoea batatas and C.longa* are efficient in inhibiting growth of S.pneumoniae with low IC_{50} values.

From the figure 5 Phytochemicals like *Ipomoea batatas*, *Curcuma longa* and *Colocasia esculenta* shown to inhibit the growth of *P.vulgaris* with zones of inhibition of about 0 mm (50 µL), 1.0 mm (60 µL) and 1.7 mm (80µL) with LD 50 value 8mg/80µL (*Colocasia esculenta) and* 0.8 mm (50 µL), 1.0 mm (60 µL) and 1.4 mm (80µL) with observed IC_{50} of 8mg/80µL with *Ipomoea batatas*. In case of *Curcuma longa* zones of inhibition formed include 0 mm (50 µL), 0 mm (60 µL) and 0.7 mm (80µL) with recorded IC_{50} value of about 8mg/80µL. High concentration of phytochemicals are required to inhibit growth of *P. vulgaris* compared to *S.pneumoniae* indicates high virulence and pathogenicity .

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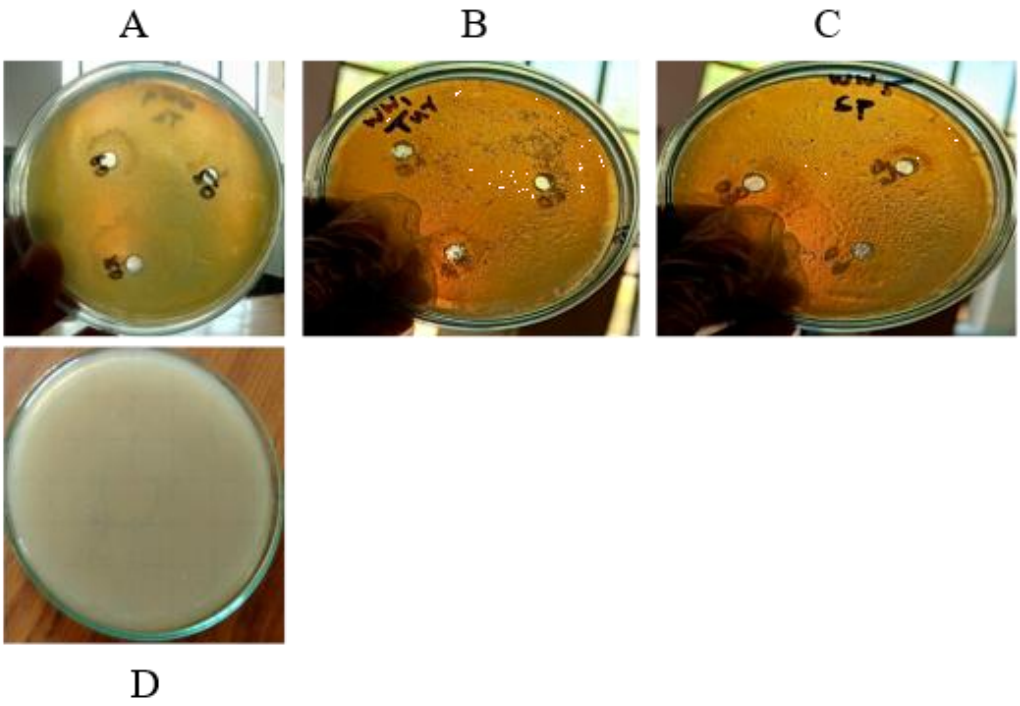

Figure:5 Antimicrobial susceptibility test on *Proteus vulgaris* with aqueous extracts of (A) *Ipomoea batatas*, (B) *Curcuma longa* ad (C) *Colocasia esculenta*

S.No	Plant Extract	Volume (µL)	Zone Diameter(mm)	IC50
1.	*Colocasia esculenta* (Taro)	50 µL	0.1 mm	8mg/80 µL
		60 µL	1.0 mm	
		80 µL	1.4 mm	
2.	*Ipomoea batatas* (Sweet Potato)	50 µL	1.6 mm	5mg/50 µL
		60 µL	1.5 mm	
		80 µL	1.3 mm	
3.	*Curcuma longa* (Turmeric)	50 µL	1.3 mm	5mg/50 µL
		60 µL	0mm	
		80 µL	1.3 mm	
4.	*Zingiber officinale* (Ginger)	50 µL	0 mm	8mg/80 µL
		60 µL	0 mm	
		80 µL	1.4 mm	

Table: 4 IC_{50} calculation based on the zones of inhibition formed by *S.pneumoniae* with phytochemical extracts of *Ipomoea batatas, Zingiber officinale, Curcuma longa* ad *Colocasia esculenta*

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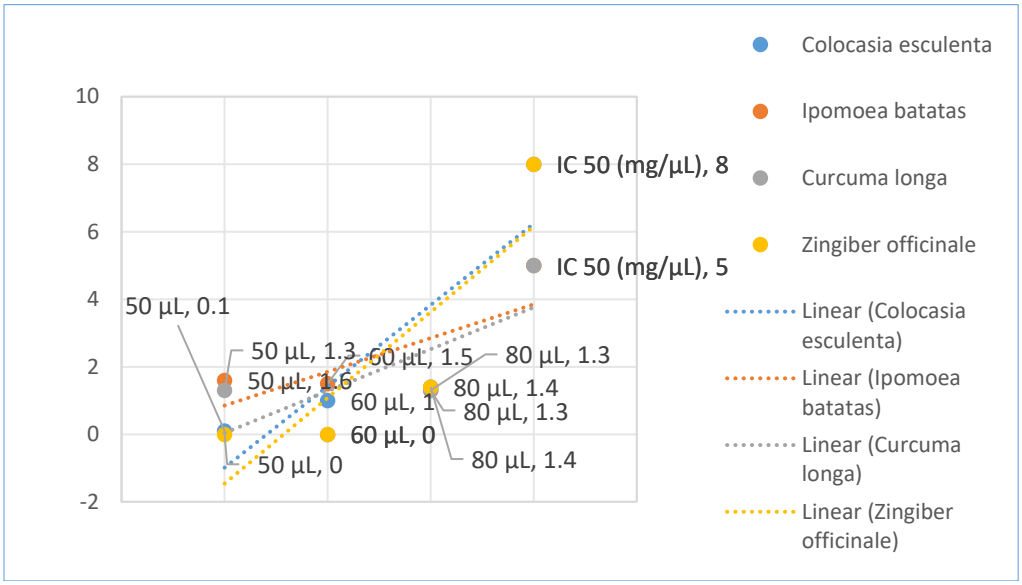

Graph: 1 Graphical representation of zones of inhibition formed by *S.pneumoniae* on agar plate and IC $_{50}$ Values.

S.No	Plant Extract	Volume(µL)	Zone Diameter(mm)	IC50
1.	*Colocasia esculenta* (Taro)	50 (µL)	0 (mm)	8mg/80 µL
		60 (µL)	1 (mm)	
		80 (µL)	1.7(mm)	
2.	*Ipomoea batatas* (Sweet Potato)	50 (µL)	0.8(mm)	8mg/80 µL
		60 (µL)	1 (mm)	
		80 (µL)	1.4(mm)	
3.	*Curcuma longa* (Turmeric)	50 (µL)	0 (mm)	8mg/80 µL
		60 (µL)	0 (mm)	
		80 (µL)	0.7(mm)	

Table: 5 IC$_{50}$ calculation of *Proteus vulgaris* based on antimicrobial susceptibility test carried using different phytochemical extracts like *Ipomoea batatas, Curcuma longa and Colocasia esculenta* and zones of inhibition formed on the agar plate

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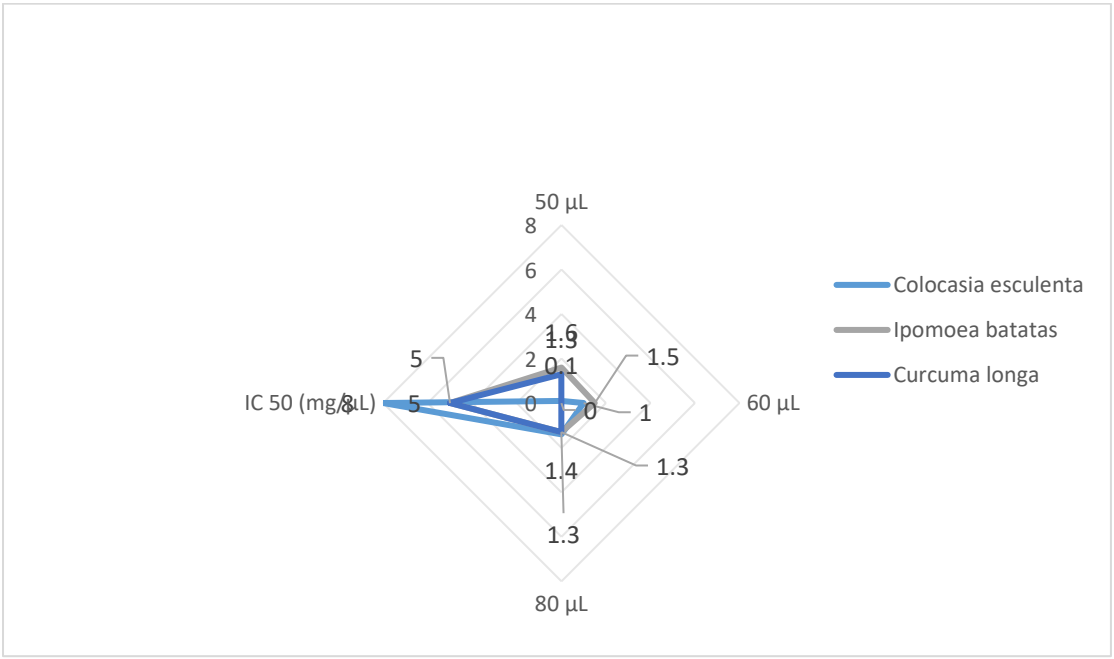

Graph:2 Radar graph representing the IC_{50} value and zones of inhibition formed by *Proteus vulgaris.*

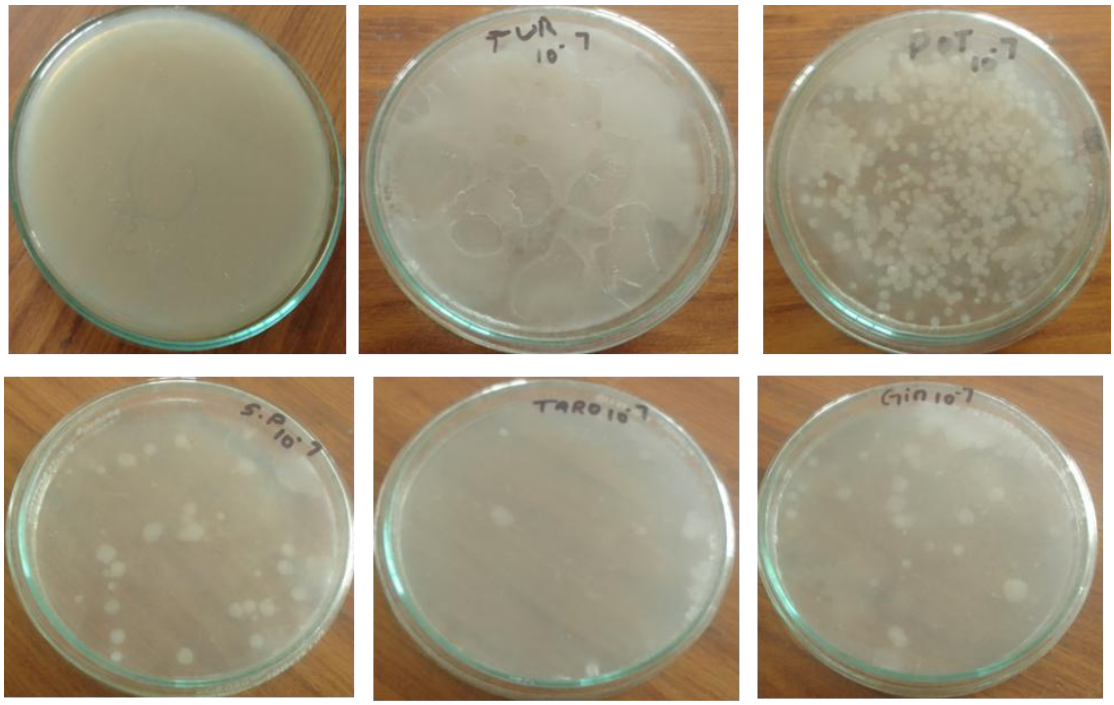

Figure: 6 Microbial Load present in the curd preserved using phytochemical extracts of *Solanum tuberosum, Ipomoea batatas, Zingiber officinale, Curcuma longa* and *Colocasia esculenta*. (Results after 1 Day of plating).

Commercial curd available in the market is used to test the preservative efficacy of phytochemicals like *Solanum tuberosum, Ipomoea batatas, Zingiber officinale, Curcuma longa* and *Colocasia esculenta* and the preservative efficacy is analyzed by studying the microbial load after preservation by spread plating technique.

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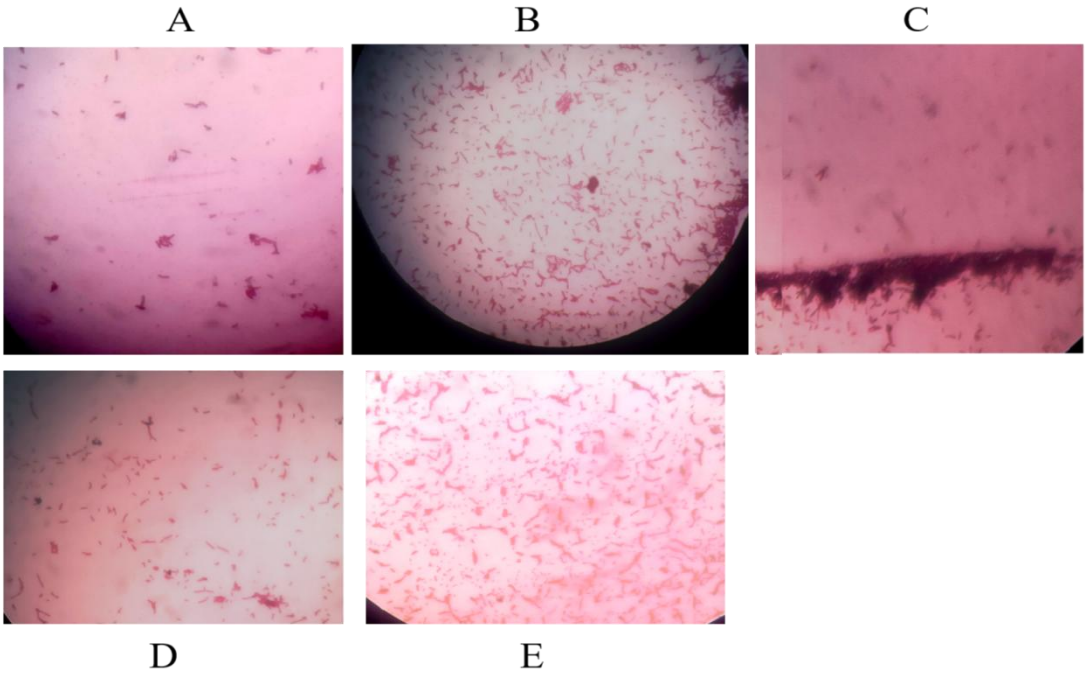

Figure: 7 Confirmation of Strain of microbial load on curd plated agar plate after preservation of 1 day by using Gram staining procedure.

From the figure 6 & 7 all the five phytochemicals *Solanum tuberosum, Ipomoea batatas, Zingiber officinale, Curcuma longa* and *Colocasia esculenta* are effective in preserving the curd for duration of 1 day and microbial load present is also proven to be low. The strain grown on agar plates is identified as *Lactobacillus* with all the five phytochemicals used in the study.

S.No	Phytochemical Used	No.of colonies
1.	*Solanum tuberosum* (Potato)	> 300
2.	*Ipomoea batatas* (Sweet Potato)	27
3.	*Zingiber officinale* (Ginger)	25
4.	*Colocasia esculenta* (Taro)	5
5.	*Curcuma longa* (Turmeric)	No individual colonies

Table: 6 Colony count on agar plate of curd preserved using phytochemicals like *Solanum tuberosum, Ipomoea batatas, Zingiber officinale, Curcuma longa and Colocasia esculenta*.

*A*lkaloids,Flavanoids, phenols and tannins present in the phytochemicals *Solanum tuberosum, Ipomoea batatas, Zingiber officinale, Curcuma longa and Colocasia esculenta* are responsible for the low microbial load and these compounds may be less effective in inhibiting the growth of lactobacillus due to lactic acid produced by the bacteria. Colony count was found to be less with all the phytochemicals except with *Solanum tuberosum* of > 300 colonies.

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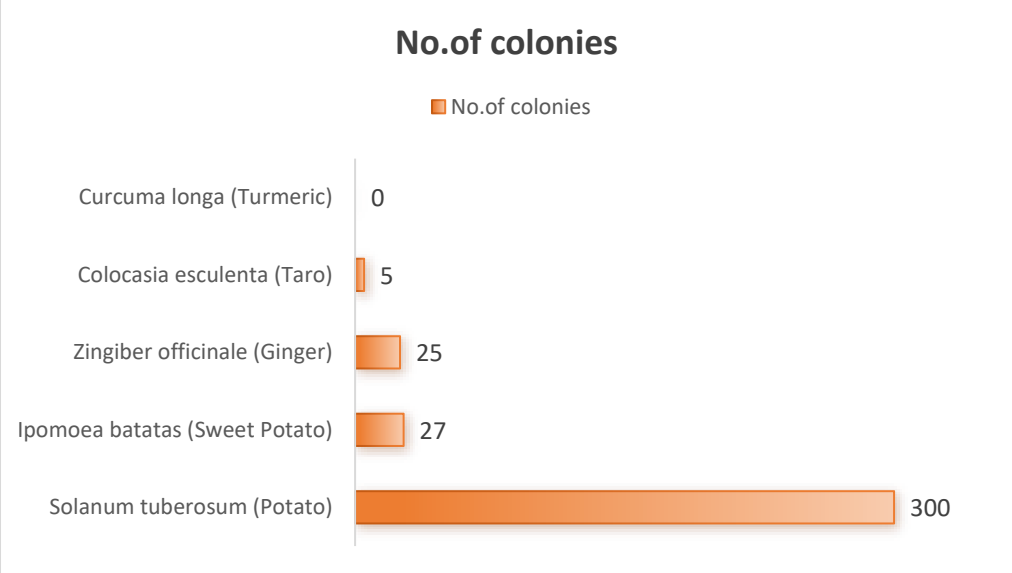

DISCUSSION AND CONCLUSION:

P. aeruginosa is an oppurtunistic gram negative pathogen that infects persons with cystic fibrosis, burn wounds, immunodeficiency, chronic obstructive pulmonary disorder (COPD), cancer, and severe infection requiring ventilation, such as COVID-19 (Qin et al., (2022)). Even after continuous efforts on understanding the pathogenicity of P.aeruginosa much has to be uncovered in terms of multidrug resistance, host pathogen interactions and mammlian signalling pathways (Qin et al., (2022)). *Proteus vulgaris* isolated from pangasius tissue samples are resistant to antibiotics like Doxycycline, Dicloxacillin, Polymyxin B, Chloramphenicol, and Imipenem exhibiting multidrug resistant phenotypes (Das et al.,(2024)).

S. pneumoniae is a pathogen that resides in nasopharynx and colonization of bacteria in lungs, blood and other tissue sites can cause pathogenic effects due to presence of proteases which can help as self protein processors and also by invading the proteins of the host precursors (Mary E Marquart (2021)). *C.freundii* is an oppurtunistic gram negative pathogen that infects infants and immuno compromised individuals causing infections like sepsis and meningitis (Jabeen (2023). Studies on *C.freundii* and its characterization proved to contain prophages that belongs to Myoviridae (48.16%), Siphoviridae (42%), Podoviridae (4.67%), Inoviridae (0.17%), and 5% of the intact prophages could not be assigned into any family (Jabeen (2023)).

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