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PHYTOCHEMICAL ANALYSIS AND PRESERVATIVE EFFICACY OF AQUEOUS EXTRACTS OF T. INDICA, C. LIMON RHIZOME AND C.LONGA AT ROOM TEMPERATURE

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Abstract:

Use of natural preservatives for preservation of canned foods and packed foods can reduce the risk of side effects and carcinogenic potential caused by chemical preservatives used for preservation. *T. indica, C.limon* peel powder and *C.longa* rhizome powder are the three natural preservatives used for the current study of preservation of Yoghurt, Wine and Apple juice. *T.indica* is effective as a preservative for both wine and yoghurt. Apple juice can be preserved by using natural preservatives *Citrus limon* peel and C.longa rhizome.

Keywords: *T. indica, C.limon, C.longa ,* Shelf life*,* Preservative efficacy

INTRODUCTION:

Curcumin, a bioactive compound found in *C.longa* is proved to contain wide range of biological properties like antibacterial, anti fungal, antiinflammatory and antiviral and well known for its antioxidant potential and can be used for preservation of canned milk and milk products (1). Sensitivity of *C.longa* is low for Multidrug resistant strains like *S. aureus, E.coli, P.mirabilis* (2) and *Proteus vulgaris* (3).Chemical constituents of *C.longa* include majorly three curcuminoids, curcumin (diferulolyl methane), Demethoxy curcumin and bisdemethoxy curcumin. Other constituents can be volatile oils like tumerone, atlantone and zingiberone, various sugars, proteins and resins (1).

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Tamarindus indica leafs were shown to contain antibacterial and antifungal properties and aqueous extracts of *T.indica* was found to be effective against *B.subtilis* than other bacterial strains including *Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Pseudomona aeruginosa* and *Candida albicans* (4). Thirty two fatty acids, two other compounds 9ß, 19-Cyclo-4 ß4, 4, 14, χ-trimethyl-5ά-cholestan- 3ß-ol, 24R-Ethyl cholest-5-en, 3β-ol and 12 essential elements viz., Arsenic, Calcium, Cadmium, Copper, Iron, Sodium, Manganese, Magnesium, Potassium, Phosphorus, Lead, and Zinc were found to be present in extracts of *T.indica* (5). Ethanolic extracts of *T.indica* were found to be effective against multi drug resistant strains like *S.aureus* and *P.aeuroginosa* (6). Lupeol is the main chemical constituent in n-hexane extract of *T.indica* leaves and associated with high free radical scavenging capacity and wound healing properties (7).

Acetone and ethanolic extracts of *C.limon* proven to show antibacterial activity against *Enterococcus faecalis* and *Bacillus subtilis* and gram negative bacteria like *Salmonella typhimurium* and *Shigella sonnei* (8). Nitric oxide scavenging activity was low in ethanolic extract of *C.limon* (9) and the biological activity of *C.limon* is mainly due to presence of phenolic compounds like flavonoids (e.g., diosmin, hesperidin, limocitrin) and phenolic acids (e.g., ferulic, synapic, p-hydroxybenzoic acids). The essential oil of *C.limon* is rich in bioactive monoterpenoids like D-limonene, β-pinene, γ-terpinene (9) and *C.limon* is well known for its antibacterial, antifungal, anticancer Properties and antioxidant potential and critical for therapeutic usage (10,11). *C.limon* leaf shown to possess anticancer properties and its anticancer property is shown by greatly affecting the cell morphology of the cancer cells (9).

2. MATERIALS AND METHODS:

2.1 Preparation of plant extracts using solvents:

2.1.1 Preparation of Hexane plant extract:

1g of *T. indica* leaf powder, *C.limon* peel powder and *C.longa* rhizome powder is added to 10ml of Hexane, mixed well and allowed to stand at room temperature for 3 days. After the time period the solution is filtered using whatsmann no.1 filter paper and used as plant extract.

2.1.2 Preparation of Aqueous plant extract :

1g of *T. indica* leaf powder, *C.limon* peel powder and *C.longa* rhizome powder is added to 10ml of distilled water, mixed well and allowed to stand at room temperature for 3 days. After the required time period the solution is filtered using whatsmann no.1 filter paper and used as plant extract.

2.1.3 Preparation of Ether plant extract :

1g of *T. indica* leaf powder, *C.limon* peel powder and *C.longa* rhizome powder is added to 10ml of Ether, mixed well and allowed to stand at room temperature for 3 days. After the time period the solution is filtered using whatsmann no.1 filter paper and used as plant extract.

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2.1.4 Preparation of Toulene plant extract :

1g of *T. indica* leaf powder, *C.limon* peel powder and *C.longa* rhizome powder is added to 10ml of Toulene mixed well and allowed to stand at room temperature for 3 days. After the incubation the solution is filtered using whatsmann no.1 filter paper and used as plant extract.

2.2 Phytochemical analysis of Plant extracts:

2.2.1 Test for Carbohydrates:

Fehling's Test:

To 1ml of plant extract few drops of Fehling's A and Fehling's B reagents are added, mix well and boiled for few minutes. Formation of reddish brown colour indicates positive result.

Benedict's test:

To 1ml of plant extract few drops of benedict's reagent is added and subjected to boiling in water bath. Formation of red colour indicates positive result

Barfoed's test:

To 1ml of plant extract few drops of barfoed's reagent is added and subjected to boiling in a water bath. Formation of brickish brown precipitate with in 1-2 min indicates positive result for monosaccharides.

2.2.2 Test for ketones:

To 1ml of plant extract few crystals of resorcinol is added and equal amount of conc. HCL is added and heated over a spirit lamp.Formation of pink colour indicates presence of ketones.

2.2.2.1 Rotherra's Prusside test:

1ml of plant extract is saturated with ammonium sulphate and 2 to 3 drops of freshly prepared sodium nitroprusside solution (0.5%) is added followed by addition of 2ml of ammonia solution. Formation of purple colour interface at the top of the solution indicates positive result.

2.2.3 Tests for Phenols and Tannins:

10% Fecl3 test:

10% Fecl3 solution is prepared and few drops of the reagent is added to 1ml of the plant extract and change in colour from reddish brown to green colour indicates positive result.

2.2.4 Tests for Alkaloids:

Wagner's test:

To 1ml of extract few drops of wagner's reagent is added and formation of yellow or brown precipitate indicates positive result.

2.2.5 Tests for Flavanoids:

Alkaloid reagent test:

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To 1ml of extract 2 to 3 drops of sodium hydroxide were added. Formation of yellow colour indicates positive result and the colour becomes colourless by the addition of Hcl.

2.3 Preparation of samples for Preservation setup:

All the Three samples Wine, Yoghurt and Apple juice were purchased from the near by stores and seggregated 50 ml each in to different beakers and preserved by adding natural preservatives for 2 days. Preheated fruit juice was cooled to room temperature after heating and preserved by addition of natural preservatives and included as fourth sample.

2.4 Preparation of Aqueous extracts of *T. indica, C.limon peel* and *C.longa* rhizome for usage as preservative:

0.1 g of *T.indica,C.limon peel* and *C.longa* rhizome powders were weighed and transferred in to separate containers and 3ml of distilled water is added to them and allowed the extract to settle to the bottom for 5 min. The upper portion of the liquid is used as a preservative for the food samples and directly added to them.

3. RESULTS AND DISCUSSION :

3.1 Phytochemical Analysis of *T.indica* :

S.No	Contents		Hexane	Aqueous	Ether	Toulene
1.	Test for Carbohydrates	1. Fehling's Test	_	_	_	_
		2. Benedict's test	_	_	_	_
		3. Barfoed's test	_	_	_	_
2.	Test for Ketone Bodies	1. Rotherra's Prusside test	+	_	_	+
3.	Test for Alkaloids		+	+	+	+
4.	Test for Phenolics		+	_	_	+
5.	Test for Flavanoids		+	+	+	+
6.	Test for Tannins		+	+	+	+
7.	Test for Vit- C		+	+	+	+

Table: 1: Phytochemical Analysis of *T.indica*, (+) = Present, (-) = Absent.

Phytochemical analysis of *T.indica* leaf extracts was carried out and found to contain polysaccharides instead of mono and disaccharides with all the solvents and presence of Ketone bodies are indicated with the hexane and toulene extracts. Alkaloids are found to be present in all the four solvent extracts Hexane, water, ether and toulene where as phenolics are found to be present in hexane extract and toulene extract. Flavanoids, Tannins and Vit- C are found to be present in all four solvents hexane, water, Diethyl ether and toulene.

3.2.Phytochemical Analysis of *Curcuma longa* :

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S.No	Contents		Hexane	Aqueous	Ether	Toulene
1.	Test for Carbohydrates	1. Fehling's Test	_	+	_	_
		2. Benedict's test	_	+	_	_
		3. Barfoed's test	_	_	_	_
2.	Test for Ketone Bodies	1. Rotherra's Prusside test	–	–	+	+
3.	Test for Alkaloids		+	+	+	+
4.	Test for Phenolics		_	+	+	_
5.	Test for Flavanoids		+	+	_	_
6.	Test for Tannins		+	+	+	+
7.	Test for Vit- C		_	_	_	_

Table : 2 : Phytochemical Analysis of *Curcuma longa*, (+) = Present, (-) = Absent.

Test for carbohydrates are carried out with extracts of *Curcuma longa* rhizome and the aqueous extract of *C.longa* found to contain monosaccharides and disaccharides and was shown positive test results with benedicts reagent and a barfoed's reagent. Ketone bodies presence was found to be positive with Ether and toulene extracts and Secondary metabolites like alkaloids and tannins presence were found to be positive with all the four extracts [Hexane, Water, Ether and Toulene]. Phenolics and flavanoids presence are found to be positive with Aqueous and Ether extracts [phenolics] and hexane and aqueous extracts [Flavanoids].

3.3 Phytochemical Analysis of *Citrus limon* Peel :

S.No	Contents		Hexane	Aqueous	Ether	Toulene
1.	Test for Carbohydrates	1. Fehling's Test	+	_	_	_
		2. Benedict's test	+	_	_	+
		3. Barfoed's test	_	_	_	_
2.	Test for Ketone Bodies	1. Rotherra's Prusside test	–	+	–	–
3.	Test for Alkaloids		+	+	+	+
4.	Test for Phenolics		+	+	+	+
5.	Test for Flavanoids		+	+	+	+
6.	Test for Tannins		+	+	+	+
7.	Test for Vit- C		+	+	+	+

Table:3 : Phytochemical Analysis of *Citrus limon* Peel, (+) = Present, (-) = Absent.

Phytochemical analysis of *C.limon* peel solvent extracts shown positive results for monosaccharides with Hexane and toulene solvents where as ketone bodies presence was found positive with aqueous and toulene solvent extracts of *C.limon*. Secondary metabolites like

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Alkaloids, Phenolics, Flavanoids and Tannins and vitamins like Vit- c are found to be present in all the four solvent extracts.and has shown positive reaction with all the tests performed for detection of secondary metabolites.

3.4. Heat treatment and Preservation of Yoghurt, Apple Juice and wine using natural preservatives like *T. indica, C. limon and C.longa :*

Use of artificial colors and preservatives to preserve the food and beverages can possess carcinogenic potential and can induce mutagenesis in humans. Natural preservatives and natural preserving methods can help in preserving the food materials with out harmful side effects produced or induced by artifical and synthetic colors generally used as food preservatives. Phytochemical analysis of *C.limon* and *T.indica* reported to contain vit-C which possess antioxidant and possess preservative properties that can increase the shelf life and also can add flavour to the food.

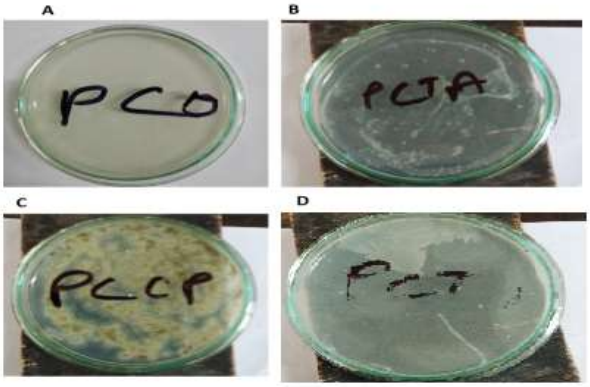

Figure: 1 : Effect of Natural preservatives *T. indica, C. limon and C.longa* on microbial growth in Yoghurt preservation and assessment of shelf life after 1 day : (A) control, (B) Effect of *T.indica* aqueous extract on microbial growth after 24 hrs of inoculation. (C) Effect of *Citrus limon* peel aqueous extract on microbial growth . (D) Effect of *C.longa rhizome* powder on microbial growth .

Studies on effect of natural preservatives *C.limon peel*, *C.longa* rhizome and *T. indica* leaf on Yoghurt preservation was carried out using aqueous extracts and *T.indica leaf extract* proved to contain anti microbial effect and preservative effect on yoghurt, there by increasing the shelf life of yoghurt . *Citrus limon* peel and *C. longa* rhizome aqueous extracts failed to inhibit the growth of microbes in yoghurt when compared to *T.indica*.

Aqueous extracts of *C.limon* peel and *C.longa* rhizome powder are found to be effective in preservation of Apple juice compared to aqueous extract of *T.indica* of concentration (100µl) used as preservative (1g/100ml W/V) at room temperature.

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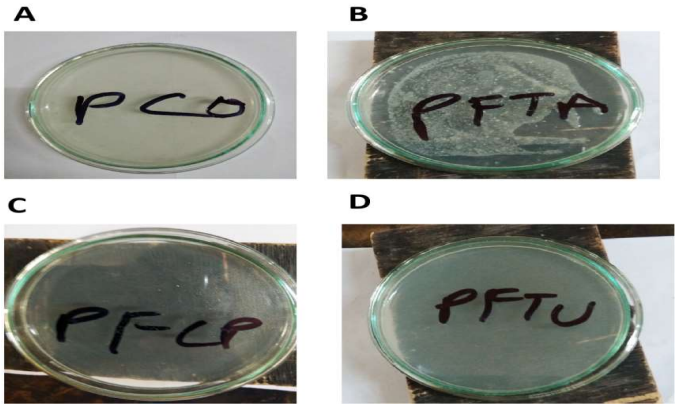

Figure: 2 : Effect of *T. indica, C. limon and C.longa* aqueous extract on Apple Juice after 24 hrs of preservation **:** (A) Control (B) Effect of *T.indica* aqueous extract on microbial growth (C) Effect of *C.limon* peel powder and (D) *C.longa* aqueous extract on microbial growth.

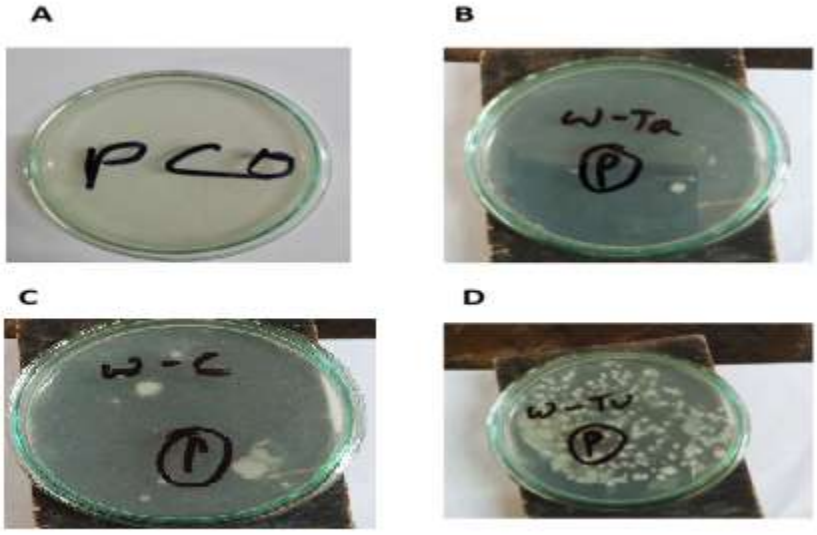

Figure: 3 : Preservation of wine using natural preservatives like *C. limon* peel, *T.indica* leaf and *C.longa* rhizome powder. (A) Control (B) Spread plate method analysis of microbial growth inhibition using aqueous extract of *T.indica*. (C) Assay of microbial growth inhibition in wine using aqueous extract of *C.limon* peel powder. (D) Effect of *C. longa* aqueous extract on preservation of wine.

Aqueous extract of *T.indica* proved to possess preservative properties on wine compared to *C.limon* peel and *C.longa* aqueous extracts. The preferred pH in wine after purification ranges up to 3.0 - 3.5 and the pH of aqueous extract of T.indica leaves was found to be slightly acidic with in the range of 6.08 - 6.38, there by preventing the growth of alkaliphiles and reducing the spoilage and taste and aroma of the wine.

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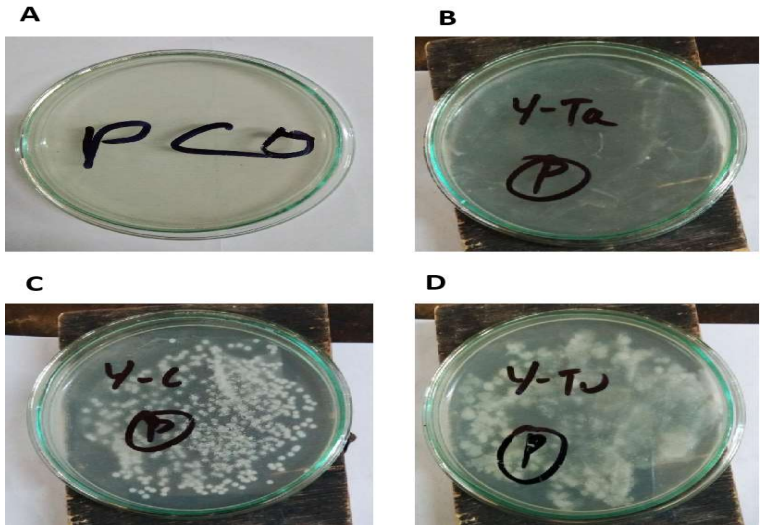

Figure: 4 : Preservation of yoghurt using aqueous extracts of *T.indica, C.limon and C.longa* aqueous extracts : (A) Control (B) Growth inhibiting activity of *T.indica* aqueous extract on yeast, Growth of Fungi in curd after addition of natural preservatives (C) *C.limon* and (D) *C.longa.*

Yeasts are major contaminants in the milk and milk products due to handling procedures and responsible for the decreased shelf life or storage period of the yoghurt. Our studies on *T.indica* majorly found to inhibit the growth of *S.cerevisiae* and the plant extract also proved to exhibit anti microbial activity on *S.cerevisiae* there by reducing the contamination by yeast.

Figure: 5 Preservation of preheated Apple juice using aqueous extracts of *T.indica, C.limon and C.longa* aqueous extracts: (A) Preheated juice added with natural preservative *T.indica*. (B) *C.longa* and (C) *C.limon* peel. (D) Control.

T. indica, C.longa and *C.limon* peel does prevent the growth of microbes in Preheated apple juice and the malic acid which is the major component in apple juice loses water molecules on heating and converts to malic anhydride which was property of promoting growth of microbes especially firmicutes by activating the functional enzymes in the bacteria.

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3.5: Determination of Microbial Growth in Curd, Yoghurt, Apple Juice and wine with out adding preservatives:

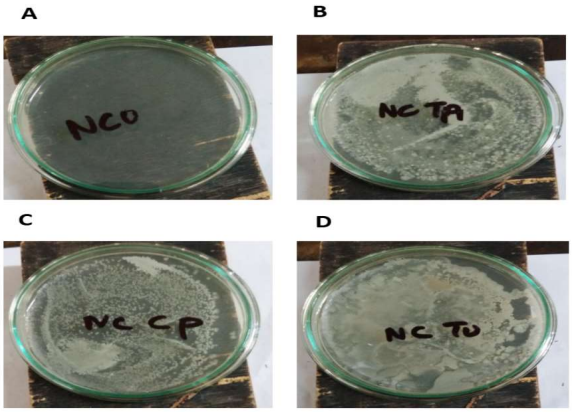

Figure: 6 : Preservation of Curd for 24hrs with no added preservatives (A) Control, (B,C,D) Yoghurt preserved for 24 hrs at room temperature with no added preservatives

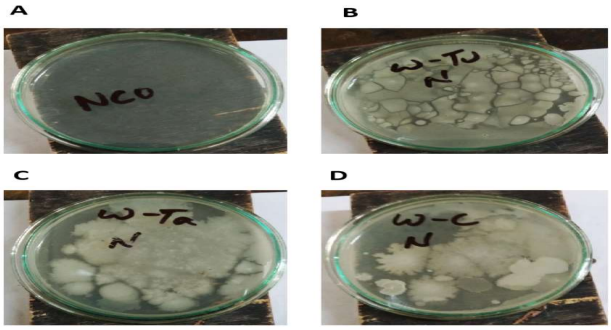

Figure: 7: Preservation of wine 24hrs with no added preservatives (A) Control, (B) wine Preserved for preserved for 24 hrsat room temperature with no added preservatives

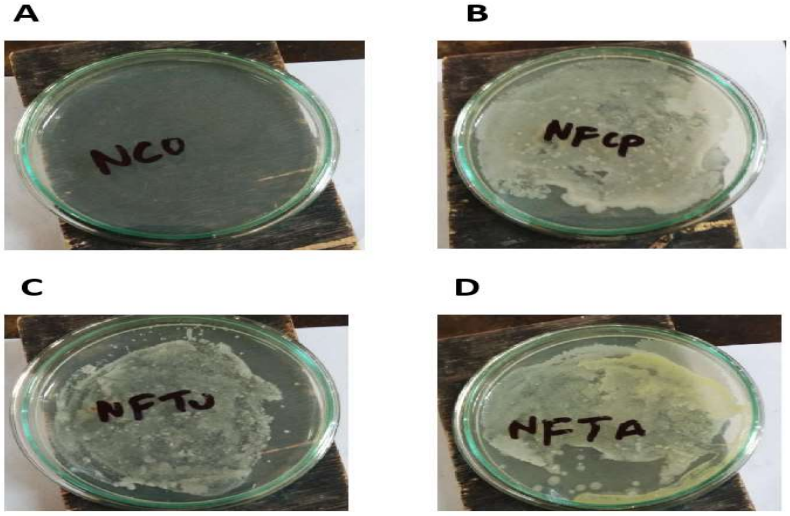

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Figure: 8 : Study of microbial growth in Apple juice after 24hrs storage at room temperature with no added preservatives . (A) Control (B) Microbial growth in Apple Juice after 24 hrs of storage with no added preservatives.

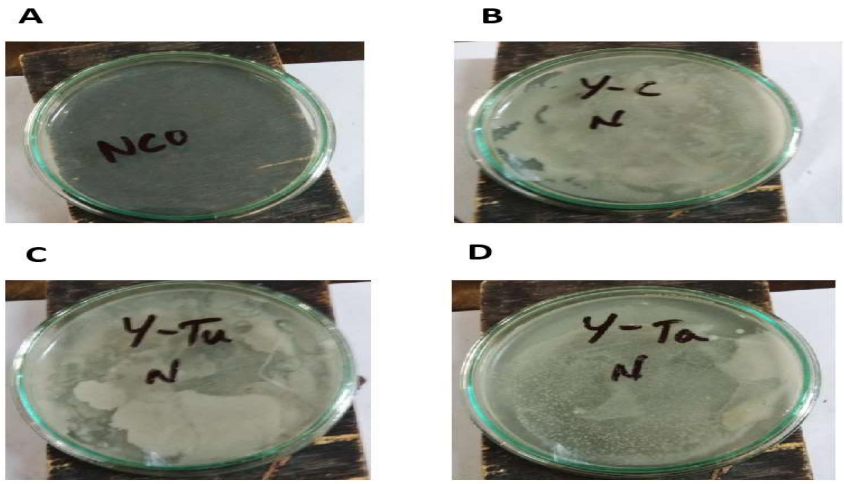

Figure: 9 : Preservation of Yoghurt at room temperature with no added preservatives. (A) Control (B,C,D) Determination of microbial count in Yoghurt after 24 hrs preservation at room temperature with no added preservatives.

Apple juice, Wine and yoghurt stored at room temperature with no added preservatives spoiled on day 2 itself due to microbial growth and showed excessive growth of bacteria and yeast on Nutrient agar and czapex dox agar plates up on inoculation.

Preservation of canned foods and food products (wine, Apple Juice and Yoghurt) using natural preservatives like *T.indica, C.limon* peel and *C.longa* can increase the Shelf life and storage period and can reduce the spoilage during transport.

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