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Synthesis And Characterization Of Medicinal Plant Extracts Loaded Pva/Pvp Blended Films And Their Applications

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ABSTRACT

Antibacterial agent based on herbal extracts is considered an attractive area for Developing countries. Nevertheless, herbal aqueous extracts usually show drawbacks, such as long-term volatility, poor bio availability and rapid burst release. For this reason the current interest of science is focused on biodegradable polymers. In this study, polymer films were prepared from poly (vinyl alcohol) (PVA) blended with poly (vinylpyrrolidone) (PVP) then post-loaded with Clitoria ternatea and Ocimum tenuiflorium ethanolic extracts. The effects of two polymers (PVA, PVP) and of the incorporated extracts were studied concerning the physical and in vitro bacterial growth inhibition properties of films; additionally, the antioxidant activity of each extracts was investigated. Plant extracts conferred significant antibacterial effects to films toward gram negative and gram positive bacteria. Also, both Clitoria ternatea and Ocimum tenuiflorium extracts showed strong antioxidant against DPPH in vitro. The prepared films showed significant antibacterial activities, specifically in films loaded with the Ocimum tenuiflorium extract against S. Aureus and in films loaded with C. Ternatea against E.coli.

Keywords: polymers, biodegradable, plant extract, antibacterial activity, antioxidant activity, plant extract, PVA/PVP film

INTRODUCTION

These days, developing nations are drawn to the antibacterial agent market based on herbal extracts. However, the majority of herbal aqueous extracts have disadvantages such fast burst release, limited bioavailability, and long-term instability. Different formulations using biodegradable and biocompatible polymers as pharmaceutical active carriers for these extracts may be utilized to address these issues[1]. Generally speaking, polymers improve antibacterial agents' therapeutic qualities by strengthening their sticky qualities, enabling the agent to function precisely at a specific location, and extending the duration of drug release by strengthening their reservation qualities[2, 3]. In many various applications, including food packaging, antimicrobial membranes for water treatment, wound dressings, and drug delivery systems, polymer films have been widely exploited as therapeutic carriers[4-6].Furthermore, a multitude of research indicate that the most significant film-forming polymers possessing water-soluble, biocompatible, and biodegradable qualities are polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP)[7].

Polyvinyl alcohol (PVA) is a multi-hydroxyl group [0-H] polymer, it has been used for medical and electrical applications[8, 9]. This is because of its high tensile strength, flexibility, and degradability. However, PVA's chemical makeup gives it a strong hydrophilic property, which enables direct interaction with the fluids of living cells[10,11]. This conduct diminishes the bioapplicability of PVA. Thus, it presents an opportunity for usage in biomedical applications when combined with other polymers, such as poly Vinyl Pyrrolidone (PVP)[12,13]. Moreover, polymer mixing creates novel materials with properties determined by their constituents[14].

Plants are important, as we all know. A growing understanding of the significance of medicinal plants has emerged in recent years, and the plant kingdom is a treasure trove of possible medications. Plant-based medications are widely accessible, reasonably priced, effective, safe, and hardly cause adverse effects[15].

Clitoria ternatea, also referred to as butterfly pea, is a perennial leguminous twinner that is a member of the Fabaceae and Sub-family Papilionaceae[16]. In ayurvedic scriptures, it is regarded as a MEDHYA RASAYANA[17]. Clitoria ternatea is also referred to as blue-pea, Kordofan, pea, and Cunha in Brazil and Sudan[18].C. ternatea is a tall, thin, climbing legume with five leaflets and deep blue flowers that has deep roots[19]. Pinnate leaves, solitary, deep blue to blue mauve blooms, flat, linear, beaked pods, olive, brown, or black seeds, and taproot roots with few branches are all present[20]. Long employed as a brain tonic, the roots, seeds, and leaves of C. ternatea are thought to enhance memory and intelligence. In India, the entire plant is used as an antidote for scorpion stings and snake bites[21]. Its wide range of phenolic compounds—flavonoids and anthocyanins, in particular—have led to its usage as a natural food and beverage color.When maintained away from direct light at low room temperature, it tends to have increased stability, making it suitable for use as a pH change indicator[22].

According to Ayurveda, Ocimum tenuiflorium, an aromatic shrub in the Lamiacea family of basil, is said to have

originated in north central India. It is referred to as "The Incomparable One," "Mother Medicine of Nature," "The Queen of Herbs," and is also called a "elixir of life." Ocimum tenuiflorium is a highly valued culinary and medicinal herb with a pleasant aroma. Ocimum tenuiflorium is an upright, fragrant shrub that grows to a height of three to five feet. Its flavor and aroma are very strong. It is the primary plant capable of storing carbon dioxide

for an astoundingly long period of time. It releases oxygen early in the morning, which is beneficial for people with respiratory disorders[23]. The plant's extracts are commonly used to treat a variety of ailments, including the common cold, irritability, intestinal illness, heart disease, headaches, stomach problems, kidney stones, heart problems, and more. It is very important in the fight against malaria. It contributes to lowering blood cholesterol levels[24].

Finding the antibacterial activity of plant extracts loaded with PVA/PVP films is the aim of this work. Additionally, the physical characteristics of PVA/PVP polymer films that are associated with the antioxidant

activity of plant extracts were also determined.

Materials and Methods

Chemicals required:

Ethanol, Polyvinyl alcohol, polyvinyl pyrrolidone, glycerol, Con.HCl, H_2SO_4

Collection and processing of plant Samples:

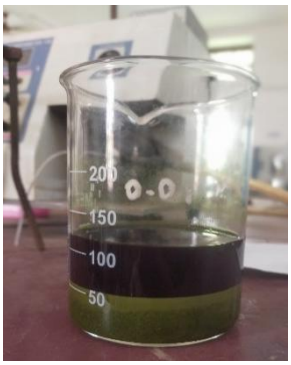
Ocimum tenuiflorium and Clitoria ternatea leaves that were healthy and free of illness were gathered, chopped into little pieces, properly cleaned in tap water, and then rinsed with distilled water until no foreign objects were left. Freshly cleaned leaves were allowed to dry for about ten days at a temperature of 25 to 28 degrees Celsius in the shade. An electronic blender was used to powder the dehydrated plant pieces. For later usage, the powdered samples were kept out of direct sunlight in an airtight container.

Extract Preparation:

Using the maceration (soaking) method, twenty grams of powdered leaf were continually extracted with ethanol in a stoppered container for 48 hours while being frequently stirred until the soluble matter was dissolved. Whatmann No. 1 filter paper is used to filter it. For additional research, the obtained ethanolic extract was employed.

Films preparation:

The solvent-casting technique was used to create the plant extract-containing films. In order to dissolve 6 grams of polyvinyl alcohol (PVA) and 6 grams of polyvinyl pyrrolidone (PVP), 100 milliliters of distilled water were used. Then, at 80°C, 10 milliliters of plant extract and 2 milliliters of glycerol were added and swirled for an hour. The mixes were put into a 20 ml Petri dish and dried for two hours in a hot air oven. The produced films were kept for the characterisation tests at room temperature (28°C). The figure depicts a representation of the film preparation.



Characterization of Films :

Absorbance, Wavelength, Structural and Surface morphology

FT-IR was used to assess the functional and spectral analysis of film samples throughout the 800-4000cm-1 range. Scanning electron microscopy was used to determine the film samples' form.

Porosity, solubility, moisture content and degradation

Porosity: Based on a prior report, the porosity of the film was ascertained using an insoluble solvent. To put it briefly, ethanol and chloroform were saturated into the films. Film samples were weighed both before and after immersion. Equation was used to calculate porosity.

$$\text{Porosity} = \frac{(m_2 - m_1)}{\rho V} \times 100$$

Where,m1 and m2 represents the weight of the film, respectively, prior to and following its immersion in ethanol and chloroform. The formula used to compute the film's volume before immersion was length x width x height, and the density of ethanol and chloroform was denoted by ρ.

Solubility: The percentage of dissolved dry matter following immersion in water was used to calculate the solubility of films. In short, the initial dry weight (Wi) of film samples of the proper size was measured after they were dried for 24 hours at 60 °C in a hot air oven. For a full day, the film samples were submerged in the necessary

volume of distilled water. Samples that had dissolved were dried for 24 hours at 100 °C in a hot air oven. in order to calculate the final dry weight (Wf). The formula was used to determine the solubility of films.

$$\text{Water solubility} = \frac{(W_i - W_f)}{W_i} \times 100$$

Moisture content: After drying at 100 °C for 24 hours in a hot air oven, the initial (Wi) and final (Wf) weights of the film samples were recorded in order to determine their moisture content. The equation was used to determine the moisture content.

$$\text{Moisture content} = \frac{(W_i - W_f)}{W_i} \times 100$$

Degradation: After seven days of degradation, the initial (Wi) and final (Wf) weights of the film samples were recorded in order to analyze their degradation. The equation was used to compute the deterioration.

$$\text{Degradation} = \frac{(W_f - W_i)}{W_i} \times 100$$

QUALITATIVE AND QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS

Test for alkaloids (Mayer's test): To a few ml of plant sample two drops of mayer's reagent are added along the sides of the test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

Test for carbohydrates (Benedict's test): To a few ml of plant sample two ml of Benedict's reagent are added and boiled. Appearance of reddish brown precipitate indicates the presence of carbohydrates.

Test for proteins (Ninhydrin Test): Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of proteins.

Test for flavonoids (Alkaline reagent test): Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for steroids: Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

Test for terpenoids: 5ml of each extract was mixed into 2 ml of chloroform, and the concentrated sulphuric acid was carefully added to form a layer. A reddish brown coloration of the interface was formed to show the positive result for the presence of terpenoid.

Test for tannins ($FeCl_3$Test): Extract was dissolved in 5ml of distilled water and treated with neutral 5% $FeCl_3$. Appearance of dark green colour indicates the presence of tannins.

Test for saponins: Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins

Test for glycosides (Salkowski's test): Crude extract was mixed with 2ml of chloroform.Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of glycoside.

Test for fixed oil (spot test): A small quantity of extract is pressed between two filter paper. Oil stain on the paper indicates the presence of fixed oils.

IN VITRO ANTIOXIDANTS ACTIVITY:

Selection of Extraction Factors

Many factors affected the extraction of antioxidants. Only three of those elements that have a significant impact on the extraction were chosen for this study. The study by Samuagam et al. (2013) states that the total phenolic content (TPC) and free radical-scavenging capacity of tropical fruit peel were examined in relation to the effects of ethanol concentration, extraction time, and extraction temperature. With ethanol concentrations ranging from 0% to 100% and the best values of DPPH, the ideal ethanol concentration was chosen for this investigation.

Determination of DPPH Radical Scavenging Activity

Singh (2014) used a modified version of the DPPH approach to measure Radical Scavenging Activity. An extract stock solution was made. In a volumetric flask, 0.01g of DPPH was dissolved in 250 ml of methanol to create a 0.004% DPPH solution. Next, 4 milliliters of DPPH solution were mixed with 1 milliliter of extract or standard ascorbic acid from the stock solution. After giving the mixture a good shake, it was allowed to stand in

the dark for half an hour. A UV-visible spectrophotometer was used to quantify the absorbance of the resultant solution at 517 nm by tracking the absorbance's decline. The standard was ascorbic acid. The following formula was used to determine the extract's scavenging activity:

Scavenging activity % = 100 × (1-AS/AC)

Where, AC is the absorbance of the DPPH solution without extract or only control, and AS is the absorbance of the solution after extracts have been added at a specific quantity.

Preparation of reagent and blank solution

By dissolving 0.01g of DPPH in 250ml of methanol in a volumetric flask, 0.004% DPPH was created. 4 ml of 0.004% DPPH was added to the test tube holding 1 ml of methanol, and it was thoroughly mixed.

Preparation of standard solution:

The ascorbic acid served as a standard or positive control when determining the level of radical scavenging activity. 2.5 grams of ascorbic acid were dissolved in 50 milliliters of methanol to create a stock solution (50 mg/ml) of ascorbic acid. To get ascorbic acid concentrations of 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0 mg/ml, aliquots were taken out of the stock solution and mixed with 20, 40, 80, and 100µL of standard ascorbic acid in different test tubes, and 980, 960, 920, and 880µL of methanol solvent, respectively. After thoroughly mixing 4 milliliters of 0.004% DPPH into each test tube containing a sample concentration, the samples were allowed to sit at room temperature for 30 minutes in the dark. Using a UV-Visible Spectrophotometer (UV-7804C), the absorbance was measured at 517 nm after 30 minutes of incubation against the blank, which was the same combination without the ascorbic acid. Two people participated in the experiment.

ANTIBACTERIAL ACTIVITY: Disc Diffusion Method

Mueller-Hinton Agar Medium:

Mueller-Hinton agar is regarded as the best media among the numerous that are available for routinely assessing the susceptibility of bacteria for the following reasons:

• It has low concentrations of trimethoprim, tetracycline inhibitors, and sulphonamide. • It exhibits satisfactory batch-to-batch repeatability for susceptibility testing. It provides most nonfastidious pathogens with sufficient development.

•A substantial amount of information and expertise has been gathered about susceptibility tests carried out using this media.

Mueller-Hinton agar is generally dependable when testing for susceptibility. Outcomes acquired from certain batches may occasionally differ greatly. Zones acquired in a disk diffusion test will typically be larger than predicted and may exceed the permitted quality control limits if a batch of media does not support proper development of a test organism. It is recommended to utilize only Mueller Hinton medium formulations that have been tested in accordance with the NCCLS Protocols for Evaluating Dehydrated Mueller-Hinton Agar and that satisfy the acceptance limitations specified therein.

Preparation of Mueller – Hinton Agar:

Preparation includes the following steps.

• Prepare Mueller Hinton agar by following the manufacturer's directions using a commercially available dehydrated base.

•A Cool it in a water bath set between 45 and 50 degrees Celsius right away after autoclaving.

•Transfer the recently made and chilled media onto glass or plastic petri dishes with a flat bottom that are level and horizontally oriented, ensuring that the depth of the medium is consistent at around 4 mm. This is equivalent to 60–70 ml of medium for 150 mm diameter plates and 25–30 ml for 100 mm diameter plates.

•If the plate is not going to be used that day, then the agar medium should be allowed to cool to room temperature and kept in a refrigerator (2 to 8°C).

• Unless sufficient measures have been taken, like wrapping in plastic, to avoid drying of the agar, plates should be used within seven days of preparation.

• Each batch of plates should have a representative sample that is incubated at 30 to 35°C for at least 24 hours in order to check for sterility.

ANTIFUNGAL SCREENING

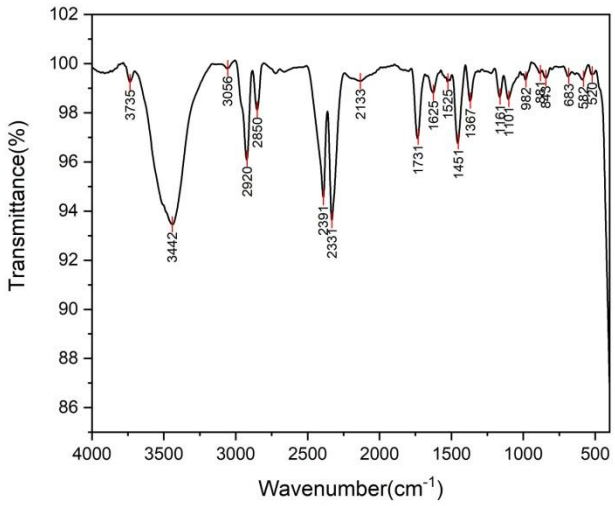
To test the ethanolic orange peel extracts' antifungal properties, the Agar well diffusion method was employed. In the middle of a sterile Petri dish, one milliliter of fungal culture was pipetted. After cooling, molten potato dextrose Aga (PDA) for fungi was added to the Petri dish holding the in column and thoroughly mixed. After solidification, sterile cork borers with a diameter of 6 mm were used to create wells in agar plates with inoculums. The corresponding wells were then filled with 100 ml of each extract (20% w/v). We chose the extract concentration (20% w/v) based on prior research and our own pre-experiments. For 30 minutes, the plates were refrigerated to allow the extracts to thoroughly diffuse into the agar. The plates were then incubated for seven days at 30°C. By measuring the zone of inhibition, which included the diameter of the well, that developed during the incubation period, antifungal activity was found. A usual 10% concentration of ketoconazole was used as an antifungal medication.

RESULT AND DISCUSSION

FT-IR spectroscopy study

Plant extracts' distinctive peaks and their functional groups were found using FT-IR spectroscopy. Based on its peak ratio, the component's functional groups were divided. Figure..... shows the FT-IR spectrum chromatogram profile of the ethanolic extract of C. ternatea and O. tenuifloruim leaf, while Table..... shows the FT-IR peak values and functional groups.

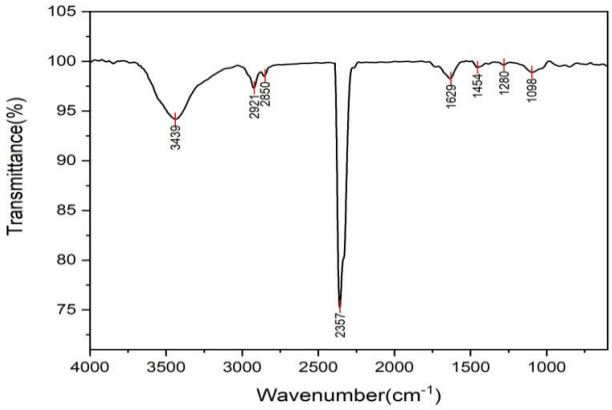
In C. ternatea, the C-O streching peak was found at 1101, the C-H bending peak at 1451, and the C=O streching peak at 1731. O=C=O stretching is seen in the absorption at 2331, whereas C-H stretching was seen at 2920 cm-1 and 2850 cm-1, respectively. 3442, on the other hand,exhibits N-H stretching.



FT-IR peak values and functional group of ethanolic extract of C.ternatea

Bond/streching	Frequency cm-1	Functional group
N-H streching	3442	Primary amine
CH	2920	Alkane
CH	2850	Alkane
	2391	
O=C=O	2331	Carbondioxide
C=O	1731	Aldehyde
C-H	1451	Alkane
C-O	1101	Vinyl ether

In O. tenuiflorium, the peak was found at 1098 in relation to C-O stretching, at 1280 in relation to C-O stretching, and at 1465 in relation to CH bending. O=C=O stretching is shown by the absorption at 2357, while C-H stretching was absorbed at 2921 and 2850, respectively. On the other hand, N-H stretching is seen in 3439.



FT-IR peak values and functional group of ethanolic extract of O.tenuiflorium

Bond/stretching	Frequency cm-1	Functional group
N-H stretching	**3439**	**Primary amine**
C-H stretching	**2921**	Alkane
C-H stretching	**2850**	**Alkane**
O=O=O stretching	**2357**	**Carbondioxide**
	1629	
CH bending	**1454**	**Aldehyde**
C-O streching	**1280**	**Alkyl arylether**
C-O streching	**1098**	**Secondary alcohol**

GC-MS analysis

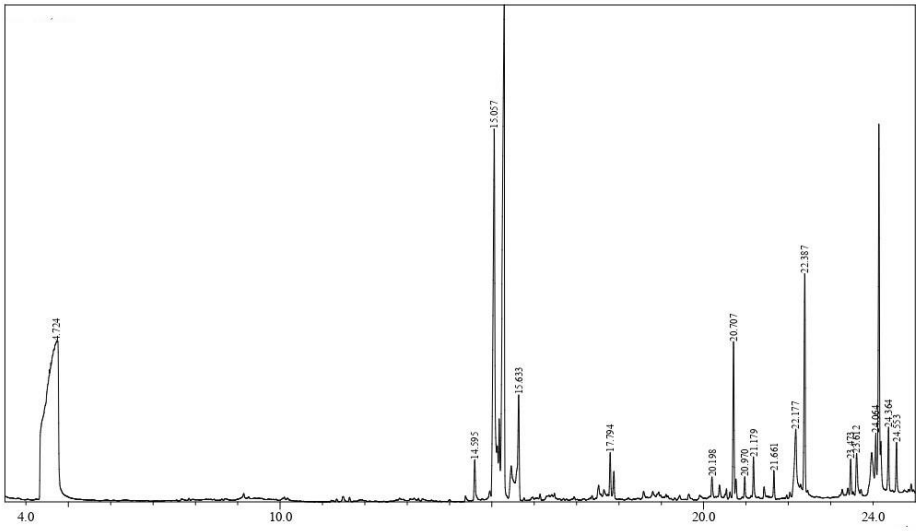
GC-MS was used to identify the various phytochemical components present in the ethanolic extract of C.ternatae and Ocimum tenuiflorium.The extract's chromatogram was displayed in figure and condensed in table.

GC-Ms chromatogram of C.terneatae leaf extract showed 12 peaks indicated the presence of 12 phytochemical constituents.

Peak	R.Time	Area	Name	Formula
1	8.649	1.66	Diethylpthalate	$C_{12}H_{14}O_4$
2.	11.183	2.13	1,6-cyclodecadiene	$C_{10}H_{16}$
3.	11.240	6.74	Iridomyrenecin	$C_{10}H_{16}O_2$

4.	11.353	4.00	E,Z-1,3,12-Nonadecatriene	$C_{19}H_{34}$
5.	11.684	41.19	9-Octadecenoicacid(Z)-,2,3-dihydroxypropylester	$C_{21}H_{40}O_4$
6.	11.883	6.21	2-Dodecen – 1- yl(-)succinic anhydride n-hexadecanoic acid	
7.	12.403	5.52	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
8	12.525	1.94	2-methoxy-4,5- methylene dioxybenzaldehyde Pentatriacontane	
9.	12.686	0.78	Pentatriacontane	$C_{35}H_{20}$
10	13.906	3.16	4-methyl – exo- tricyclo [6.2.1.0(2.7)]undecane	$C_{12}H_{20}$
11.	14.095	23.24	9-Octadecenoic acid, (E)	$C_{18}H_{34}O_2$
12.	14.294	3.42	6, Octadecenoic acid, (Z)	$C_{18}H_{34}O_2$

GC-MS chromatogram of O.tenuifloriumleaf extract showed 17 peaks indicated the presence of 17 phytochemical constituents.



Peak	R.time	Area %	Name	Formula
1	4.724	52.38	Dimethylsulfoxoniumformylmethylide	$C_4H_8O_2S$
2	14.595	1.33	Phenol, 2-methoxy-3-(2-propenyl)	$C_{10}H_{12}O$
3	15.057	17.47	Benzene,1,2-dimethoxy-4-(1-propenyl)	$C_{11}H_{14}O_2$
4	15.633	3.54	Caryophyllene	$C_{15}H_{24}$
5	17.794	1.12	Dodecanoic acid, ethyl ester	$C_{14}H_{28}O_2$
6	20.198	0.59	Octadecanoicacid, ethyl ester	$C_{20}H_{40}O_2$
7	20.707	4.08	Neophytadiene	$C_{20}H_{38}$
8	20.970	0.54	3,7,11,15-Teyramethyl-2-hexadecen- 1-ol	$C_{20}H_{40}O$

9	20.179	1.10	3,7,11,15-Teyramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$
10	21.661	0.80	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
11	22.177	3.55	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
12	22.387	6.57	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$
13	23.473	0.97	9-Octadecenoic acid(Z)-, Methyl ester	$C_{19}H_{36}O_2$
14	23.612	1.60	Phytol	$C_{20}H_{40}O$
15	24.064	1.14	Linoleic acid ethyl ester	$C_{20}H_{36}O$
16	24.364	1.83	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$
17	24.553	1.41	2-Hexadecen-1-ol,3,7,11,15-teyramethyl-,acetate,[R-[R*,R*-(E)]]-	$C_{20}H_{40}$
		100.00		

SEM Assay

Figure __ presented the surface morphology of C.ternatea and O.tenuiflorium respectively.

A homogenous appearance was observed on the both surface of the films. It shows miscible blending between PVA and PVP. On the otherhand, the film demonstrated rough surface.The rough surface is due to the distribution of C.terrnatea and O.tenuiflorium extract molecules over the exising interconnecd microporous spaces of the film network.

Solubility, Moisture Content

The solubility and moisture content for O.tenuiflorium and clitoriaterneatea films are present in **table.....**

Film property	**C.ternatea**	**O.tenuiflorium**
Solubility	-189.26	-170.99
Moisture content	17.24	10.256

After a day, it showed a reduction to films O.tenuiflorium (-170.99) exhibits lower solubility than C.terneatea (-189.26).The hydrophilic properties of PVA and PVP may be the cause of this Comparably, C.terneatea has a higher moisture content (17.24) in comparison to O.tenuiflorium (10.256). Following the addition of the extract, a little rise that was brought about by intermolecular hydrogen bonding with water molecules was visible.

Degradation:

The degradation test for O.tenuiflorium and C.terneatea films are present in table.....

Film property	**C.ternatea**	**O.tenuiflorium**
Degradation	80.76	5.22

It demonstrated the degradation of films after 7 days. The C.ternatea shows greater degradation (80.76) than O.tenuiflorium(5.22).

Qualitative and quantitative analysis of phytochemicals

The distribution of different phytochemical constituents in ethanolic extracts of powder of leaf of C.ternatea and O.tenuiflorium. was evaluated qualitatively and summarized in table

Secondary metabolites	Clitoria ternatea	Ocimum tenuiflorium
Alkaloids	-	+
Carbohydrates	+	-
Proteins	-	+
Flavanoids	-	+
Steroids	+	-
Terpenoids	-	+
Tannin	+	+
Saponins	-	-
Glycosides	+	-

Note: '+'present, '-' absent

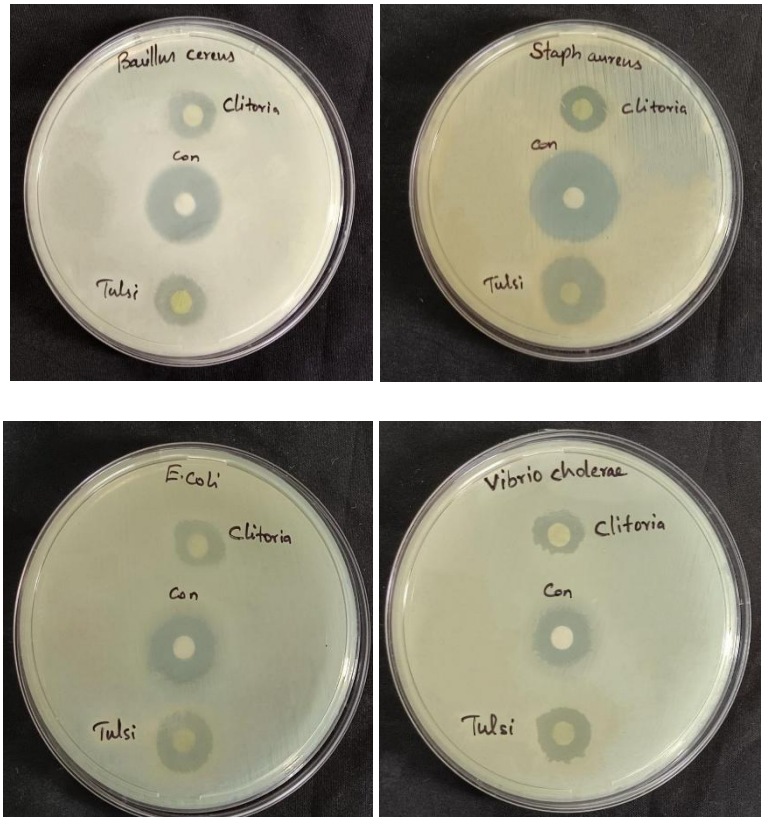
Table: Ocimum tenuiflorium and Clitoria ternatea secondary metabolites were screened phytochemically. The table made it clear that while C. Ternatea lacks protein, flavanoids, terpenoids, saponins, and fixed oils, C. Ternatae contains a wide range of active chemicals such as alkaloids, carbohydrates, steroids, tannins, and glycosides.
Ocimum tenuiflorium contains alkaloids, proteins, flavanoids, terpenoids, tannins, and fixed oils. Ocimum tenuiflorium lacks steroids, glycosides, saponins, and carbohydrates.

Antibacterial activity

By measuring the inhibition zone of ethanolic plant extracts of Clitoria ternatea and tulsi, which results from the active extract components penetrating through the agar, the antibacterial activity was demonstrated. It is stated in millimeters (mm). Gram positive (Bacillus cereus, Staph aureus) and gram negative (E. coli, Vibrio cholera) bacteria were used to test the antibacterial activity.

Bacteria	Clitoria ternatea	Ocimum tenuiflorium	Control
Bacillus cereus	15 mm	15.6 mm	22 mm
Staph aureus	14mm	20mm	25mm
E.coli	15.2mm	16mm	15mm
Vibrio cholera	14mm	15.4 mm	15mm

The zone of inhibition for E. coli in Clitoria ternatea is 15.2 mm, while the zone of inhibition for Staph aureus and vibrio cholera is 14 mm. The zone of inhibition for vibria cholera is 15.4 mm, while the zone of inhibition for E. coli is 20 mm for Ocimum tenuiflorium. This demonstrates that E. Coli is more sensitive in an ethanolic extract than the other microorganisms.

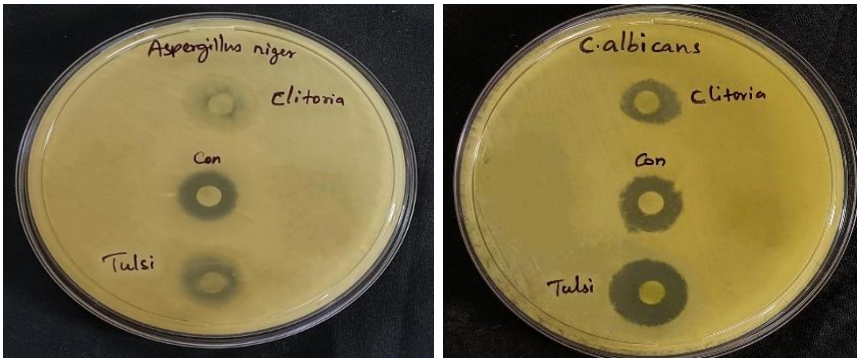


Antifungal activity

Due to the active extract components penetrating through the agar, the inhibitory zone of ethanolic plant extracts of Clitoria ternatea and Ocimum tenuiflorium was determined, indicating the antifungal activity. It is stated in millimeters (mm). Using Aspergillus niger and Candida albicans, the antifungal activity was conducted.

Fungi	**Clitoria ternatea**	**Ocimum tenuiflorium**	**Control**
Candida albicans	14.2 mm	17.3 mm	15mm
Aspergillusniger	10 mm	16.2mm	14mm

The highest zone of inhibition for Candida albicans in Clitoria ternatea is 14.2 mm, while the highest zone for Candida albicans in Ocimum tenuiflorium is 17.3 mm. This demonstrates that in an ethanolic extract,Ocimum tenuifloriumis more sensitive than Clitoria tenatea compared to the zone of inhibition.



Antioxidant activity

Concentration.....	**C.ternatea**	**O.tenuiflorium**
20	36.62± 0.004	46.60± 0.005

40	41.82±0.003	53.68± 0.001
60	48.56±0.005	57.68± 0.006
80	52.57± 0.010	65.68± 0.005
100	63.21± 0.005	70.74± 0.004
IC5O.....	64.67	30.55

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