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Formulation and Evaluation of an Phyto-Gel Blend of Typha Domingensis, Turmeric and Aloe Vera for Enhanced Antioxidant, Antimicrobial, Antifungal Efficacy.

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KEYWORDS
Typha domingensis, antioxidant, antimicrobial, curcumin, aloe vera, phyto-gel.

Abstract

Background: Typha domingensis, a perennial aquatic plant native to the Americas, is known for its antioxidant properties and antimicrobial effects. Its leaves, flowering parts, seeds, rhizomes, stems, and leaves have been used in traditional medicine for wound healing, weaving, and crafts. The plant's male and female sections prevent bacteria and fungi growth, while curcumin and aloe vera possess antibacterial, antifungal, and antioxidant properties.

Methodology: Phyto-gels are herbal gel formulations that combine various components and active constituents to provide specific effects, such as antibacterial, anti-inflammatory, and wound-healing properties. These gels are popular in wound healing due to their promotion of physiological processes and hastening the healing process. The formulation process involves extracting the herbal extract, choosing excipients, gelling agents, and preservatives.

Results and discussion: Various methods are employed in the production of polyherbal gels, with the dispersion method being the most effective. Physical attributes such as appearance, pH, spreadability, viscosity, and homogeneity are assessed, along with chemical attributes like flavonoid tests. Antimicrobial tests were conducted using the disc diffusion method (Kirby Bauer method) against E. coli and B. subtilis, and the antioxidant test was executed by Hydroxyl radical scavenging activity using the Fenton method with a minor amendment.

Conclusion: In summary, Typha domingensis, Curcumin, and Aloe vera are combined to create an antiseptic Phyto-gel, promoting synergistic effects of antifungal, antimicrobial, and reducing oxidative stress.

1. Introduction:

Antioxidants, antifungal, and antimicrobial agents play crucial roles in promoting health and combating diseases. Antioxidants neutralize free radicals, which can damage cells and contribute to aging and various diseases. Consuming foods rich in antioxidants reduces the risk of chronic diseases and overall improved health. Antibacterial agents fight bacteria and prevent infections by killing or inhibiting their growth.

Antifungal activity in plant extracts can be influenced by the components in the extract and the target fungus. Some chemicals found in plant extracts can disrupt the cell membrane, inhibit cell wall synthesis, interfere with fungal enzymes, induce oxidative stress, and interfere with fungal signaling pathways. These compounds play pivotal roles in modern medicine and healthcare, contributing to disease prevention, treatment, and overall well-being.

Typha domingensis, commonly known as southern cattail, exhibits antioxidant properties due to its high content of phenolic compounds and flavonoids. Typha domingensis has shown antimicrobial activity against various microorganisms, including bacteria, fungi, and viruses, indicating its potential as a natural antimicrobial agent.

Antifungal properties have been demonstrated in Typha domingensis extract, which has significant antifungal action against the common fungal infection Candida

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albicans. The biological source of Typha domingensis is the plant itself, which belongs to the Typhaceae family.
Typha domingensis has antioxidant properties due to its high content of phenolic compounds and flavonoids, which help neutralize harmful free radicals in the body, reducing the risk of chronic diseases. Its antifungal properties and potential uses in healthcare and medicine require further investigation.Traditional medical practices have described therapeutic applications of Typha domingensis, including staminate flowers, female flowers, seeds, rhizomes, stems, wastewater treatment, crafts, and ecological restoration. The plant's ability to absorb nutrients and filter pollutants from water makes it a valuable resource for various applications.[1,2]
Curcumin, a hydrophobic plant, has been shown to have antibacterial, antifungal, and antioxidant properties. Aloe vera, a plant from the Liliaceae family, contains water, mucilage, vitamins, minerals, enzymes, and other compounds. Its pulp has various physical and chemical properties, including wound healing, anti-inflammatory, moisturizing, anti-aging, and antiseptic effects.
A combination of these three plants: Typha domingensis, Curcumin, and Aloe vera has been utilized to create an antiseptic phyto-gel since they each have strong antioxidant and antibacterial and antifungal properties. Phyto-gels are a type of herbal gel formulation that combines various components and active constituents to provide specific effects, such as antibacterial, anti-inflammatory, and wound-healing properties. These gels are popular in the area of wound healing, as they promote physiological processes and hasten the healing process. The rationale behind antioxidants, antifungal and antimicrobial activity lies in their roles in promoting health and combating diseases.

2. Materials And Methods:

Materials: Ingredients used Typha Domingensis whole plant collected from Manchar (Pune) , Curcumin dried Rhizomes Purchase from market, fresh Aloe vera leaves , Methanol, Water, Glycerin, Guar Gum, Preservatives. and Equipments used, Soxhlet apparatus, Beakers, Stirrers, Weighing machine, Burners, Test tubes, Spatula, Petri dishes, Autoclave Platinum wire loop, Spreader, Incubator, Oven, Pipettes, Containers. All equipments are from Sterli8ng Institute of Pharmacy,Nerul, Navi Mumbai.

Methods:

Extraction of Typha domingensis, Curcumin and Aloevera [3][4]

The aerial part (leaves) of Typha domingensis Pers. was collected in January from Pune and the plant was correctly identified and authenticated in the Department of Botany, Blatter Herbarium at St. Xavier's College, Mumbai, the T.domingensis specimen provided matches with the Blatter Herbarium specimen R-1147 of R.R. Fernandez. After gathering the leaves, they were cleaned under running water and let to dry naturally. For two to four weeks, the plant components were dried in the shade. The active substances in plant leaves will be destroyed if direct sunlight is present, hence precautions were taken to avoid it.The plant leaves were dried, then ground into a fine powder and kept in an airtight container. The 50g of air-dried leaf powders were progressively extracted using methanol as the solvent in a soxhlet extraction procedure. After drying, the extracts were kept for further use in a sterile container. Extraction of Curcumin By maceration Process and anad Aloe vel gel collected from their leaves by using simple process.

Preformulation process

Flavonoid test [5][6]

A) Shinoda Test:

Fill the test tube with the alcoholic sample of T.domingensis extract. Add five to ten drops of strong HCl (hydrochloric acid). Finally, add 0.5 g of magnesium turnings. Take note of the color shift: A Reddish pink or brown colour denotes the presence of flavones.

B) Alkaline Reagent Test:

Fill a test tube with 2 ml of the T.domingensis extract. Add a few drops of the alkaline reagent, 10% sodium hydroxide (NaOH) solution.Observe the reaction: the solution shows an increase in the intensity of yellow color. After that, add a few drops of diluted HCl (hydrochloric acid) to the same mixture. The alkaline environment produced by NaOH is neutralized by adding HCl. Keep an eye out for any variations in the colour or luminosity.
Examine the outcomes: After adding NaOH, deep yellow fluorescence which would become colourless /reduce in yellow fluorescence on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Phenol test [7]

To find out if phenols are present, use the $FeCl_3$ phenol test. The following steps are included in the test: Mix ethanol and water to dissolve the sample. Drops of a diluted ferric chloride ($FeCl_3$) solution should be added.

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There are phenols present if the sample takes on a red, green, purple, or blue coloration.

Antioxidant test [8][9]

With a small modification, the Fenton technique was used to carry out the hydroxyl radical scavenger activity: -

After carefully mixing 0.2 mL of sample solutions (T.domingensis methanolic extract) at various concentrations (0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml, and 0.5 mg/ml), 1 mL of $FeSO_4$ solution (0.15 mM), 0.4 mL of H_2O_2 solution (2 mM), 0.4 mL of distilled water, and 1 mL of salicylic acid ethanol solution (6 mM) were all thoroughly mixed and allowed to sit at 37 °C for one hour.

At 510 nm, the absorbance was measured.Every reagent—aside from the sample—was taken as a positive control, and distilled water served as the control. Using Equation (1), the hydroxyl radical scavenging activity was determined.

%Radical scavenging activity (RSA) = (Absorbance of control-Absorbance of sample)/Absorbance of control*100

The identical process was carried out with ascorbic acid as a standard antioxidant sample at different concentrations (0.2, 0.3, 0.4, and 0.5 mg/ml), and its hydroxyl radical scavenging activity was also calculated using above equation.

Antibacterial test [10][11]

- **Prepare the test samples:**

  a) Preparing the test samples by using just T.domingensis extract

Take the methanolic extract of T. domingensis. The plant extract in methanol was made in the stock solution at the maximum concentration of 25 mg/ml (the stock concentration). By gradually diluting sterile distilled water to a concentration of 3.125 mg/ml, 6.25 mg/ml, and 12.50 mg/ml the various sample were prepared.

b) Preparing test samples by combining T. domingensis with curcumin & aloevera

To generate the new samples, different concentrations of T. domingensis (3.125 mg/ml, 6.25 mg/ml,12.50 mg/ml & 25 mg/ml) were mixed with 1 mg/ml concentrations of curcumin and aloevera.

- **Prepare the sample disk**

Sterile paper discs (disks) should be dipped into samples of various concentration (3.125 mg/ml, 6.25 mg/ml,12.50 mg/ml & 25 mg/ml) of Typha domingensis extract and its combination samples with curcumin & aloe vera. Additionally, dip a few disks in each of the extracts of curcumin and aloe vera. The samples should be kept properly sterile for one hour. Along with this also dip some paper disk in solvent used(methanol) for negative control.

- **Agar plate preparation**

Prepare the Agar Mixture: 100 ml of distilled water were combined with 2.8 grams of nutritional agar. Stir the mixture and heat it until all the ingredients are completely dissolved. To sterilize the dissolved mixture, autoclave it for 15 minutes at 121°C.

Cool the Agar-Let the nutritional agar cool, but do not allow it to solidify. When you pour it into the plates, it should be between 45 and 50 degrees Celsius.

Transfer the Agar Plates- Transfer around 15 to 20 ml of the nutrient-rich agar into every sterile petri dish. To guarantee that the agar is distributed evenly, gently swirl the plates. Allow the agar to fully solidify. Label and Store: Put date & time on the plates and label them properly. Until they are needed, keep the agar plates in a cold, dark area.

- **Preparation of inoculum**

For the inoculum, nutritional broth was made with beef extract, peptone, and sodium chloride. *S. aureus* and *E. coli* were utilized as the test microorganism.

- **Inoculate Agar Plates:**

Using a sterile swab and sterile conditions, equally distribute the microbiological inoculum of *S. aureus* and *E.coli* onto four agar plates each.

- **Apply the sample disk**

Utilizing a forceps and operating in sterile conditions, place each sample-loaded disc onto the agar plates that have been inoculated with S. aureus and E. coli. Additionally, arrange the positive control (Chloramphenicol) and negative control (methanol).

- **Incubate Plates:**

The plates should be incubated at the proper temperature for the chosen bacteria for 24 hrs.

- **Check for Zones of Inhibition**:

Following incubation, check the plates for regions that are clear around the discs, or zones of inhibition, which are signs of antimicrobial activity.

- **Measure Zones:**

Using a calibrated ruler, determine the diameter of the zones of inhibition.

- **Data analysis**

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It involves comparing the zones of inhibition of Typha domingensis, Aloevera, and curcumin extract samples, as well as their combination with control compounds found in both E. coli and S. aureus agar plates.

Antifungal test (by disk diffusion method)

- **Prepare the test samples:**

a) Preparing the test samples by using just T.domingensis extract

Take the methanolic extract of T. domingensis. The plant extract in methanol was made in the stock solution of the concentration of 25 mg/ml

b) Preparing test samples by combining T. domingensis with curcumin & aloe vera

To generate the new samples, T. domingensis (25 mg/ml) were mixed with 1 mg/ml concentrations of curcumin and aloevera.

- **Prepare the sample disk:**

Sterile paper discs (disks) should be dipped into samples of Typha domingensis extract and its combination samples with curcumin & aloe vera. Additionally, dip a few disks in each of the extracts of curcumin and aloe vera. The samples should be kept properly sterile for one hour. Along with this also dip some paper disk in solvent used(methanol) for negative control.

- **Agar plate preparation**

Prepare the Agar Mixture: 100 ml of distilled water were combined with 2.8 grams of nutritional agar. Stir the mixture and heat it until all the ingredients are completely dissolved. To sterilize the dissolved mixture, autoclave it for 15 minutes at 121°C. Cool the Agar-Let the nutritional agar cool, but do not allow it to solidify. When you pour it into the plates, it should be between 45 and 50 degrees Celsius. Transfer the Agar Plates- Transfer around 15 to 20 ml of the nutrient-rich agar into every sterile petri dish. To guarantee that the agar is distributed evenly, gently swirl the plates. Allow the agar to fully solidify. Label and Store: Put date & time on the plates and label them properly. Until they are needed, keep the agar plates in a cold, dark area.

- **Preparation of inoculum**

For the inoculum, nutritional broth was made with beef extract, peptone, and sodium chloride *Candida albicans* were utilized as the test microorganisms.

- **Inoculate Agar Plates:**

Using a sterile swab and sterile conditions, equally distribute the microbiological inoculum of *Candida albicans*.

- **Apply the sample disk**

Utilizing a forceps and operating in sterile conditions, place each sample-loaded disc onto the agar plates that have been inoculated with *Candida albicans*. Additionally, arrange the positive control (Cholarmphenicol) and negative control (methanol) disks.

- **Incubate Plates:**

The plates should be incubated at the proper temperature for the chosen fungi for 24 hrs.

- **Check for Zones of Inhibition**:

Following incubation, check the plates for regions that are clear around the discs, or zones of inhibition, which are signs of antimicrobial activity.

- **Measure Zones:**

Using a calibrated ruler, determine the diameter of the zones of inhibition.

- **Data analysis**

It involves comparing the zones of inhibition of Typha domingensis, Aloevera, and curcumin extract samples, as well as their combination with control compounds(Chloramphenicol) found in *Candida albicans* agar plates.

Formulation process

By using dispersion method the phyto-gel was prepared

Ingredients

Sr.no.	Ingredients	Qty (in %)	Qty (for 10g)
1	Typha domingensis extract powder	10%	1g
2	Turmeric extract powder	5%	0.5g
3	Aloe Vera gel	20%	2g
4	Guar gum	0.5%	0.05g
5	Preservative (Vitamin E)	1%	0.1g
6	Glycerine (Emollient)	5%	0.5g
7	Water	q.s	q.s

Table 1: Formulation Table

Procedure for Phyto-gel formulation

Following are the instructions used to make the phyto-gel:

Slowly stir in 5 ml of water to dissolve the guar gum. If necessary, gently reheat. Permit the mixture to become thick and hydrate.Include the about 2 grams of aloe vera gel in the guar gum solution. Mix well until completely combined.Stir the extracts of Typha domingensis and turmeric into the gel base gradually. Depending on the desired outcomes, change the concentrations.To improve emollience, add 0.5 grams of glycerine.Add 0.1 grams of vitamin E to the mixture.Stir continuously until a uniform dispersion is achieved (homogenizer/stirrer used for proper mixing). Make sure to properly label and

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provide storage instructions while packaging the polyherbal gel in appropriate containers (glass/ propylene tube).

Evaluation process of phytol-gel

pH: 1.0 g gel is accurately weighed and dispersed in 100 ml purified water. The digital pH meter that is previously calibrated with a standard buffer solution at 4.0, 7.0, and 9.0, is used to measure the pH of the dispersion [12]

Spreadability: Spreadability is determined by applying 0.25 grams of phyto-gel on a glass slide, followed by the placement of the glass slide. After five minutes with a 100g weight on the upper glass slide, the diameter of the circle spreading is finally measured.[13]

Viscosity: The viscosity of the formulated gel is determined using viscometers like Brookfield Rheometer which was run at 60 r.p.m for 1 minute at 26ºC.[14]

Homogeneity: The formulated product is inspected visually for its appearance and the presence of any aggregates after the gel had been filled in the container for homogeneity testing. They were tested.[15][16]

Skin Irritation and Patch Test: The patch test involves selecting healthy human volunteers and applying a small amount of phyto-gel to a designated area of the skin, usually the upper back or forearm. A control group with the gel base is used. The tests are left in place for 48 hours, observing for signs of irritation or adverse reactions.[17]

Washability: Apply a little quantity of gel on a healthy volunteer's hand, then wash it off and note how easy or difficult it is to remove.

Stability: Physical stability of the herbal gel was carried out for 4 weeks at various temperature condition like 2°C,25°C, and 37°C[18]

3. Results And Discussion

Extraction yield

(yield)%= (We/Wt)100

where, We - weight of completely dried extract of Typha domingensis(5g).

Wt - weight of leaves of T. domingensis plant which is undertaken for extraction(50g).

Y% = (5g/50g)100

Thus, from 50g of the T. domingensis plant's leaves, 10% of the total yield was extracted.

Preformulation test

Flavonoid test

Shinoda Test: A reddish-brown colour was observed denoting the presence of flavones.

Alkaline Reagent Test: After adding NaOH, deep yellow fluorescence which become colourless /reduce in yellow fluorescence was observed on addition of few drops of dilute Hydrochloric acid, indicating the presence of flavonoids.

Phenol test

Samples takes on a green/ reddish purple coloration, indicating presence of phenols.

Antioxidant test

a) Hydroxyl Radical Scavenging Activity of sample (T.domingensis plant extract)

%RSA was calculated using,

Calculation of % Radical Scavenging			
Absorbance measured data			
Concentration (ug/ml)	**Control**	**Standard**	**%RSA**
0.2	1.5081	1.419	6.2790
0.3	1.5081	1.405	7.3380
0.4	1.5081	1.382	9.1244
0.5	1.5081	1.379	9.3618

Table 2 :%RSA of standard (Ascorbic acid)

Calculation of % Radical Scavenging			
Absorbance measured data			
Concentration (ug/ml)	**Control**	**Sample**	**%RSA**
0.2	1.5081	1.410	6.9574
0.3	1.5081	1.391	8.4184
0.4	1.5081	1.389	8.5745
0.5	1.5081	1.370	10.0802

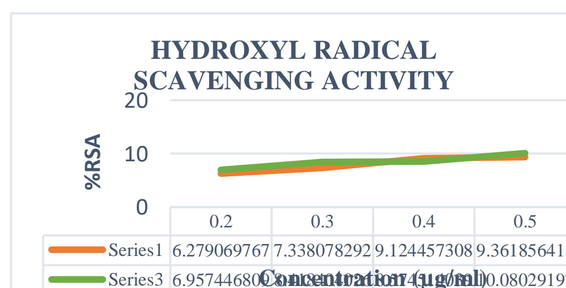

Table 3 :%RSA of sample T.domingensis

Figure 1: Antioxidant activity comparison graph between std & sample

T.domingensis antioxidant(%RSA) activity is compared to that of ascorbic acid(the standard antioxidant) both exhibit comparable activity.

Evaluation process of phytol-gel

Appearance: It has a granular gel like consistency and a translucent appearance with a greenish colour reflecting

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the natural colour of the *T. domingensis* as one of its key ingredients.

pH: The pH of the formulation was determined in order to be sure that the formulation does not shows any irritancy to the skin. The pH was found to be 6.5 ± 0.5, which was ideal for a topical preparation.

Spreadability: The diameter of this phyto gel formulation on glass slide was found to be 7.5 Cm. It indicated that phyto gel is easily spreadable by small of shear and easily applicable.

Viscosity: The viscosity was found to be 480 cP on Brookfield viscometer which was run at 60 r.p.m for 1 minute at 26°C.

Homogeneity: No aggregates were observed after filling the gel in container

Skin Irritation and Patch Test: No irritation & adverse reaction was observed after applying the gel on a hand of healthy volunteer after 24 hrs.

Washability: The gel was easily washed off just by water without/with very slight yellow tint which can be washed off by using soap.

Stability: Thus, the gel is stable at 2-8 degree C.

CONDITIONS	DAYS	RESULT
ROOM TEMPERATURE (25-27 DEGREE CELCIUS)	4 WEEKS	FUNGAL GROWTH
FRIDGE (2-8 DEGREE CELCEIUS)	4 WEEKS	NO FUNGAL GROWTH
HOT AIR OVEN (40 DEGREE CELCEIUS)	4 WEEKS	FUNGAL GROWTH

Table 4 :-Stability

Antibacterial activity

Bacteria	**Extract**	**Concentration in µg/ml**				**Positive control (Chloramphenicol)**	**Negative control (solvent)**
		3.125	**6.25**	**12.50**	**25.00**		
		Inhibition zone in mm					
E.coli	T.domingensis	9	9.5	10	11	12	6
	Turmeric	8	8.5	9	10		
	Aloe vera	5	5.5	6	6.5		
	T.domingensis phyto-gel	9.4	9.5	10	11		
	T.domingensis +Turmeric+Aloe vera phyto-gel	10.5	11	12	12.5		
S.aureus	T.domingensis	9	9	9.5	10.5		
	Turmeric	8	8.5	9	10		
	Aloe vera	5.5	6	6	6.5		
	T.domingensis phyto-gel	9	9.5	10	10.5		
	T.domingensis +Turmeric+Aloe vera phyto-gel	10	10.5	11	11.5		

Table 5 :-Inhibition zone by bacteria

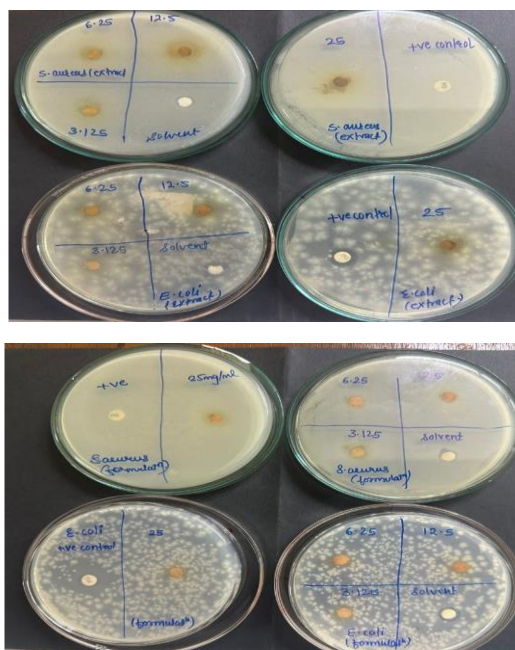

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Figure 2 :-Inhibition zone by bacteria

As T.domingensis, aloe vera, and turmeric are examined for their individual antibacterial activity and their combination, the combination shows more antibacterial action against E. coli than against S. aureus. As well as there is increase in activity with the increment in the concentration of T.domingensis.

Antifungal activity

Fungi	Extract	Concentration in µg/ml				Positive control (Fluconazol)	Negative control (solvent)
		3.125	6.25	12.5	25		
		Inhibition zone in mm					
Candida albicans	T.domingensis	9	9.5	10.5	11	11	6
	Turmeric	8	8.5	9	10		
	Aloe vera	6.5	7	8	8.5		

	Extract						
	T.domingensisphyto-gel	9.4	9.5	10	11		
	T.domingensis+Turmeric +Aloe vera phto-gel	10	10.5	11	12		

Figure 3 :-Inhibition zone by fungi

As T.domingensis, aloe vera, and turmeric are examined for their individual antifungal activity and their combination, the combination shows more antibacterial action against Candida albicans. As well as there is increase in activity with the increment in the

concentration of T.domingensis

4. Conclusion

The phyto-gel showing combined activity of antioxidant, antibacterial and antifungal was developed using 10% *T. domingnesis* 20% aloe vera gel and 5% curcumin along with other excipient. *Typha domingensis*, a native aquatic plant from the Americas, belonging to family *Typhaceae*, is known for its antioxidant qualities and antifungal & antibacterial properties due to the presence of flavonoids & phenolic phytoconstituents. Its stems, rhizomes, seeds, leaves, and blooming sections are used in traditional medicine, including wound healing. Derived from the turmeric plant, curcumin possesses hydrophobicity along

with antifungal, antibacterial, and antioxidant characteristics. Aloe vera, derived from *Aloe barbadensis Miller*, is a plant known for its antibacterial, hydrating, anti-inflammatory, and wound-healing properties, containing antiseptic substances like salicylic acid, urea nitrogen, phenols, and cinnamic acid.

The authentication & extraction of *T.domingensis* leaves (soxhlation in methanol) and selection of excipients, gelling agents (guar gum), and preservatives are steps in the formulation process. The formulation process used the most efficient approach, dispersion. Together with evaluating the phyto- gel's chemical properties, such as the flavonoid test (Alkaline reagent test & Shinoda test) & phenol's test with a positive result. The Fenton technique was modified to conduct an antioxidant test using hydroxyl radical scavenging activity (absorbance was determined at 510 nm, comparing it with a standard antioxidant, revealing a comparable antioxidant activity. The disc diffusion method (Kirby-Bauer method) was used to conduct the antibacterial test against *E. coli* and *S. aureus* as well as antifungal test against *Candida albicans* at different concentrations (25, 12.5, 6.25, and 3.12 mg/L) of *T.domingensis* as well as in combination with curcumin and aloe vera gel. In the antibacterial and antifungal test the combination of *T.domingensis* with aloe vera & turmeric have shown maximum synergistic effect as compared to individual. This phyto-gel show maximum antibacterial & antifungal activity against *E. coli* & *Candida albicans* respectively. Finally, an evaluation was conducted on the physical attributes of the phyto-gel, including its appearance, pH (6.5), spreadability (7.5 cm), viscosity (480 cP), washability (easy washable), skin irritation (no irritation after 24 hours), homogeneity (no aggregates). In addition, the phyto-gel's stability at three different temperatures—2–8 °C, at room temperature, and 40 ºC was monitored for four weeks. Based on these observations, it was determined that the phyto-gel was stable at 2–8 °C. Following the conclusion of all testing, the phyto-gel was put into a well-labelled, sterile, wide-open glass container.

As a result, T.domingensis, Curcumin, and Aloe vera have been blended to create an antiseptic phyto-gel that encourages the synergestic effects of antioxidant, antifungal, and antibacterial properties. Further clinical study is required as nearly all of this phyto-gel's physical characteristics were assessed in a lab setting.

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