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Antimicrobial Activity Of Emulgel Containing Ethanolic Extract Of *Tridax procumbens*.

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ABSTRACT

Herbal Medicines has been a base of Indian Systems of Medicines.The side effects and toxicities of synthetic antibiotics justifies the need to search new antimicrobial agents from plant source. With this objective, we thought it worthwhile to investigate the antimicrobial potential of the leaves of *Tridax procumbens* which has been used in folklore medicine. The leaves of this plant including other aerial parts except flowering tops have been claimed to be useful in the treatment of inflammatory conditions and have tendency to heal wound, anti-diabetic activity, anti-arthritic activity, preventing hair loss, diarrhoea and serve as insect repellent. The present study was to formulate & evaluate the Emulgel by using ethanolic extract of Tridax procumbens. The Emulgel was formulated for its better absorptivity and enhancing its bioavailability. The TP extract and TP emulgel was examined against various wound infecting bacteria like such as Bacillus Subtilis, E. coli and fungi like such as Candida albicans. The anti-microbial activity of *Tridax procumbens* was performed by using Kirby Bauer agar well diffusion assay method. The result obtained showed effective inhibition of anti-microbial activity by TP emulgel compared to TP extract.

Keywords: *Tridax procumbens* Linn, Antimicrobial activity, Bacteria, Fungi, Agar well diffusion method.

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Tridax procumbens

Tridax procumbens Linn. belongs to Asteraceae family [1]. It is commonly known as 'Ghamra' and in English popularly called 'coat buttons' because of the appearance of flowers. The plant has been extensively used in Ayurvedic system of medicine for various disorders. This is dispensed for "Bhringraj" by some of the practitioners of Ayurveda. It is found throughout India, it is native of tropical America and naturalized in tropical Africa, Asia, and Australia. This plant widely distributed and it's each and every part having noble pharmacological activity. The work done till to date on its pharmacological activities like hepatoprotective effect, immunomodulating property, promising wound healing activity, antidiabetic, hypotensive effect, antimicrobial, insect repellent activity, anti-inflammatory and antioxidant, bronchial catarrh, dysentery, diarrhoea also prevent falling of hairs and leads to hair growth promotion [2].

Figure 1: *Tridax procumbens* var. procumbens [3]

Scientific Classification [4]

Kingdom	:	Plantae
Division	:	Spermatophyta
Subdivision	:	Angiospermae
Class	:	Dicotyledonae
Subclass	:	Cotyloideae
Order	:	Asterales
Family	:	Asteraceae
Common name	:	Coat buttons
Botanical Name	:	*Tridax procumbens* Linn

Synonyms [4]

- Coat buttons
- Mexican daisy
- Tridax daisy

Preparation Of *Tridax procumbens* Extract

The plant *Tridax procumbens* were collected from the surroundings of Dhanalakshmi Srinivasan College of Pharmacy, Perambalur, Tamil Nadu-India. The plant is authenticated from Dr. Senthil Kumar, Head of Botany Department, St.Joseph College, Tiruchirappalli. The leaves from the plant are separated and shade dried. The dried leaves are then coarsely powdered and kept in well closed container. About

40gm powdered leaves was taken in Soxhlet apparatus, it was extracted using ethanol. The temperature was maintained between 70-40°C for 24 hours. The collected extract was then concentrated by evaporating the ethanol. Now the crude extract of *Tridax procumbens* was obtained.

Figure 2: Extraction of *Tridax procumbens* using Soxhlet Apparatus

Formulation Of *Tridax Procumbens* Emulgel

Formulation of gel

Accurately weighed Carbapol 940 was taken and dispersed in beaker containing 300ml of distilled water. The beaker was set aside for half an hour for allowing Carbapol 940 to swell. Methyl paraben sodium was dissolved in water. The Carbapol 940 was stirred continuously until no lumps are found. Then add 5-6 drops of Triethanolamine and methyl paraben sodium to the Carbapol 940 and stir continuously until a clear, transparent gel is formed.

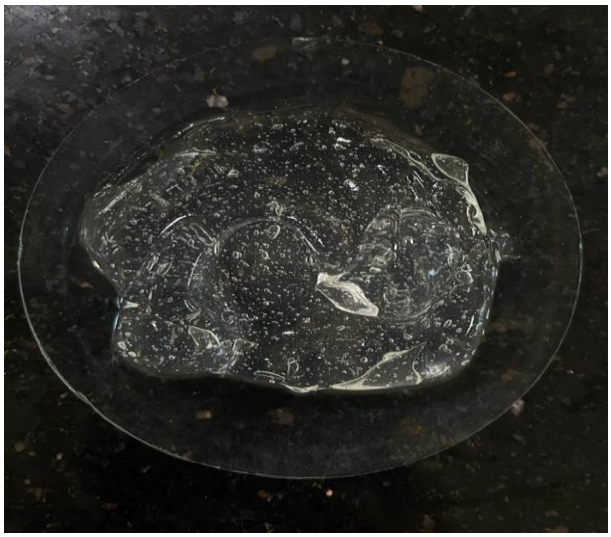

Figure 3: Gel Base

Formulation of emulsion

The oil phase of the emulsion was prepared by dissolving Span 80 in Light liquid paraffin and the extract of *Tridax procumbens* was added and heated to 70°C. Methyl paraben was dispersed in oil phase. The aqueous phase was prepared by dissolving Tween 80 in purified water and heated to 80°C. The oil phase was slowly added to aqueous phase with constant stirring until stable emulsion was formed.

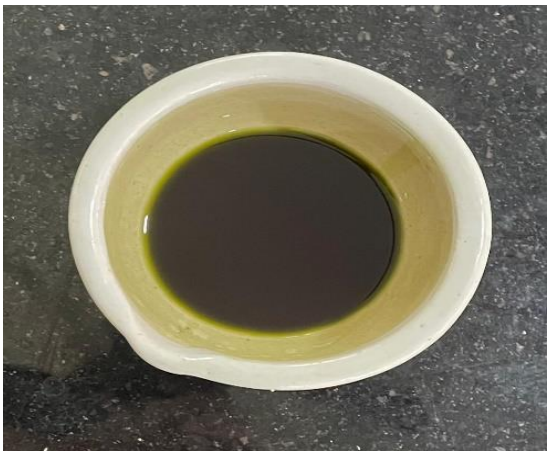

Figure 4: Emulsion of *Tridax procumbens*

Formulation of Emulgel

The gel and the emulsion containing the extract of *Tridax procumbens* was mixed in 1:1 ratio and gently stirred to form the Emulgel.

Figure 5: Emulgel contain *Tridax procumbens* extract

METHODS AND MATERIALS

Microorganisms And Culture Media

Bacterial cultures such as *Bacillus subtilis, E. coli,* and fungal culture *Candida albicans*were obtained from Eumic analytical Lab and Research Institute, Tiruchirappalli. Bacterial strains were maintained on Nutrient agar slants (Hi media) at 4°C

Inoculum Preparation

Bacterial cultures were sub cultured in liquid medium (Nutrient broth) at 37˚C for 8h and further used for the test (10^5-10^6CFU /ml). These suspensions were prepared immediately before the test was carried out.

Preparation Of Culture Media

Nutrient Agar Medium

Nutrient agar medium is one of the most commonly used medium for several routine bacteriological purposes:

Ingredients	Grams/Litre
Peptone	5gm
Beef extract	3gm
Agar	15gm
Sodium chloride	5gm
Yeast extract	1.5gm

Table 1: Ingredients for preparation of cultural media

After adding all the ingredients into the distilled water, it is boiled to dissolve the medium completely and sterilized by autoclaving at 15 Ib psi pressure (121˚C) for 15 minutes. The pH of the medium was 7.

Nutrient Broth

The nutrient broth was prepared by the same composition without agar. After adding all the ingredients into the distilled water, it is boiled to dissolve the medium completely and sterilized by autoclaving at 15 Ib psi pressure (121˚C) for 15 minutes.

Assay Of Antimicrobial Activity

Microbial inoculum preparation

The nutrient broth was prepared. The identified bacterial colonies were inoculated into the broth culture and were used for antimicrobial activity.

Kirby Bauer Agar Well Diffusion Assay

The *in-vitro* antimicrobial activity was conducted by agar well diffusion method. This method is based on diffusion of antimicrobial component from the reservoir hole to the surrounding inoculated agar medium so that the growth of microbe is inhibited as zone around the hole.

The nutrient agar medium was prepared and sterilized by autoclaving at 121˚C 15 lbs. pressure for 15 minutes. It was aseptically poured into the sterile petri plates and was allowed to solidify. The Bacterial broth culture was swabbed on each Petri plates using a sterile bud. Then wells were made by well cutter at 10mm diameter in the agar media spread with microorganism. The organic solvent extracts of leaves were added to each well aseptically.

This procedure was repeated for each petri plates. Then the petri plates were incubated at 37˚C for 24 hrs. After incubation the plates were observed for the zone of inhibition.

RESULTS AND DISCUSSION

The prepared TP Emulgel of F1 & F2 formulation exhibited for antimicrobial activity against various microorganism causing wound infecting such as *Bacillus Subtilis, E. coli and Candida albicans.*

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Anti-Bacterial Activity Of F1 And F2 Formulation

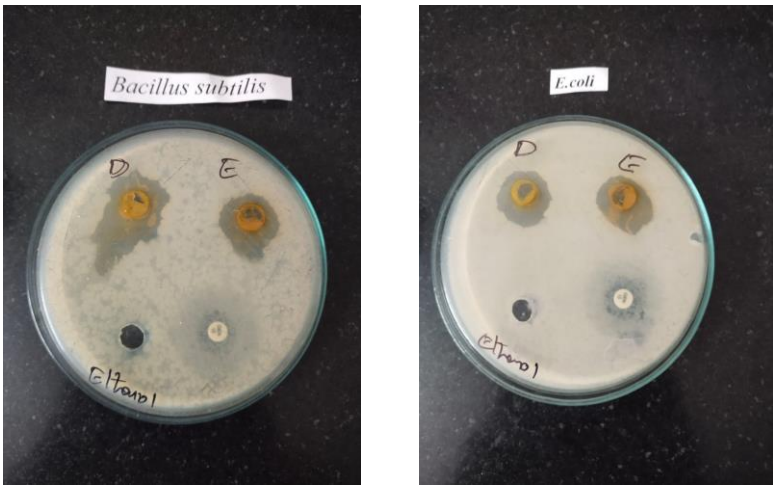

Figure 6: Antibacterial activity of TP Emulgel, TPE, Control, Ethanol

D- Emulgel Formulation containing 2mg of TP Extract
E- Emulgel Formulation containing 1 mg of TP Extract

Sample	Sample Concentration 100 µl added and Zone of inhibition (mm/ml)				
	D/F2 (2mg)	**E/F1(1mg)**	**Extract**	**Control (Amoxicillin)**	**Ethanol**
Bacillus Subtilis	25	20	11	20	0
E. coli	28	20	12	20	0

Table 2: Antibacterial activity of TP Emulgel, TPE, Control and Ethanol

The Antibacterial activity of crude TPE and emulgel containing two different concentrations of TPE was done on Bacteria like Bacillus Subtilis & E. coli. It shows that the Zone of inhibition was found to be 11 for crude TP Extract whereas F1 shows 20 and F2 shows 25. It clearly states that the Formulation F2 shows the highest range of zone of inhibition and has high antibacterial activity compared to formulation F1 and extract.

Anti-Fungal Activity Of F1 And F2 Formulation

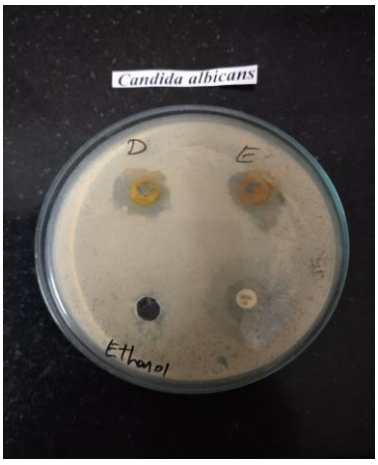

Figure 7: Antifungal activity of TP Emulgel, TPE, Control, Ethanol

D-Emulgel Formulation containing 1mg of TP Extract
E- Emulgel Formulation containing 2mg of TP Extract

Sample	Sample Concentration 100 µl added and Zone of inhibition (mm/ml)				
	D/F2 (2mg)	E/F1(1mg)	Extract	Control (Amoxicillin)	Ethanol
Candida albicans	32	24	13	22	0

Table 3: Antifungal activity of TP Emulgel, TPE, Control, Ethanol

TheAntifungal activity of crude TPE and emulgel containing two different concentrations of TPE was done on fungi *Candida albicans*. It shows that the Zone of inhibition was found to be 13 for crude TP Extract whereas F1 shows 24 and F2 shows 32. It clearly states that the Formulation F2 shows the highest range of zone of inhibition and has high antifungal activity compared to formulation F1 and extract

CONCLUSION

The formulated Emulgel containing TP extract determines the good anti microbial activities. This could make them potential topical anti microbial agents effective in the treatment of skin infection. The use of ethanolic TP extract produces an effective anti microbial property. In the present study, the ethanolic extract of TP and Emulgel showed effectively inhibited the growth of all selected bacterial and fungal species. The TP Emulgel showed more effective inhibition against the wound infecting bacteria like such as *Bacillus Subtilis, E. coli* and fungi *Candida albicans* compared to ethanolic extract of TP. So, the prepared Emulgel have better Anti microbial property. The anti- bacterial & antifungal activity of the Formulation F2 shows higher zone of inhibition when compare to the extract alone &F1 formulation. By these results we found that the formulation of TP Emulgel F2 shows good anti microbial activity. The wound healing activity will be further confirmed by Animal Studies (*in vivo*) in Future.

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