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Formulation and Evaluation of *Tridax Procumbens* (L.) L. Herbal Soaps

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ABSTRACT

Introduction: Traditional medicine is an important source of potential therapeutic compounds. *Tridax procumbens* has great importance in traditional medicine because of its good antibacterial and antifungal properties. The current research aims to formulate herbal soap using methanolic extract of T. procumbens leaves and evaluate its physicochemical properties.

Method: The herbal soaps are formulated using T. procumbens leaf extract. The leaves were extracted by the Soxhlet extraction method using methanol as solvent. The plant extract was evaluated for phyto constituents like saponins, phenols, alkaloids, flavonoids, tannins and steroids. Four different formulations are formulated with varying doses of plant extract and ingredients. The physical parameters like colour, odour, appearance and evaluation parameters like pH, moisture content, % alcohol insoluble matter, foam height, foam retention and % free alkali are evaluated for four formulated soaps.

Results: The plant extract consists of phyto constituents like saponins, phenols, alkaloids, flavonoids, tannins and steroids. All four formulations had good appearance, uniform colour and odour.

Conclusion: Among four soap formulations, F1 soap had the least number of impurities whereas F3 soap had stable foam. The evaluation parameters are in the limits prescribed by Bureau of Indian standards. Hence formulated soaps can further be standardised and used.

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Introduction

Skin is the first line of defence in the human body. The skin constitutes 15% of the total body weight, as it is the largest organ. It protects against physical, biological and chemical attacks and plays an important role in thermoregulation by preventing the loss of excess water from the body (Kolarsick et al., 2011). Crores of bacteria, fungi, and viruses reside on our skin and constitute the skin microbiota. These act as a physical barrier to prevent the invasion of pathogens. When the balance between these symbiotic bacteria and pathogens is disturbed, it may result in skin infections (Allyson et al., 2018). For many years skin problems are common ailments that are affecting humans (Akuaden et al., 2019). Common causes of skin infections are the invasion of the skin by pathogenic microorganisms. So, any substance with antimicrobial properties, which either kills the microorganism or inhibits the growth of the microorganism is essential for treating skin infections.

Nature has been a major source of herbs with immense antimicrobial potential to treat mild to severe types of skin diseases. Many ancient medical systems like Ayurveda, Siddha and Unani systems of medicine have explored the use of several herbal preparations for treating skin infections (Christudas et al., 2012). According to the World Health Organisation reports, Traditional medicine is fulfilling the primary health needs of 80% of the population. The dependency on traditional medicine is more in developing countries. The increasing desire to investigate new potential drugs in natural sources has led us to create some amazing medicines (Policepatel & Manikrao, 2013).

Herbal formulations with antifungal and antibacterial activities can be prepared from various parts of the plant like stem, leaves, roots, bark, flower, or fruit for skincare. These medicines can be applied topically or administered orally. For topical administration, these medicines are formulated in the form of cream, lotion, gel, soap, sap, or ointment (Kareru et al., 2010). Herbal soaps are one of the highly used formulations for skincare and for treating skin diseases.

Soap is a surface-active agent and it is chemically the alkali metal salt of long-chain fatty acids. When a fat or oil containing triglycerides is reacted with alkali, soap is formed by a reaction called saponification reaction (Akuaden et al., 2019). Generally, soaps are prepared by the melt and pour method, hot press method and cold press method. Oils like coconut oil, palm kernel oil, olive oil, castor oil, sunflower oil, rice bran oil and soybean oil among others are used for soap preparation. The quality of soap depends on the type of oil used, type of alkali used, its hardness, foam height, moisture content, and total fatty matter. (Sindhu et al., 2019). Herbal soaps incorporated with herbal extracts should show significant antimicrobial activity, provide conditioning to the skin, have good foam, fragrance and are gentle on the skin.

One of the potential plants with antimicrobial properties is *Tridax procumbens* (L.) L (*T. procumbens*). It is generally called coat buttons in English, jayanthi veda in Sanskrit, balapaku or gaddi chamanthi in Telugu. It belongs to the family Asteraceae. *T. procumbens* is a widely spread herb, covered with hair (Jain, 2012). It is widely spread in India up to 2400 m above sea level even though it is primarily native to tropical regions of South and North America (Vinod & Nagaraju, 2015).

T. procumbens has great importance in traditional medicine. It is used to treat colds, inflammation, anaemia and liver diseases in Central America. In Guatemala, it is used as an antibacterial, antifungal, and antiviral agent and plays an important role in the treatment of vaginitis, stomach pain, diarrhoea, mucosal inflammations, and skin infections. In India, leaf juice is used to treat wounds and bleeding. Gastrointestinal and respiratory infections, high blood pressure, and diabetes are some of the ailments for which *T. procumbens* is used significantly. The whole plant is used for the treatment of protozoal infections, like malaria, leishmaniasis and dysentery in Guatemala (Beck et al., 2018). In the West African sub-region and tropical region of the world, the leaves of the plant are used as a remedy for conjunctivitis by traditional practitioners and the native people (Pandey & Tripathi, 2014).

The methanolic extract of the leaves consists of phytoconstituents like alkaloids, flavonoids, phenols, saponins, steroids, and tannins (Kushwaha et al., 2018). The plant is rich in minerals like iron, copper, zinc, sodium and other trace minerals like phosphorus, potassium, selenium, calcium and magnesium (Vinod& Nagaraju, 2015). Phytoconstituents like luteolin, kaempferol, apigenin, catechins, myricetin, biochanin A, baicalein, quercetin, phenolic acids, vanillic acid, akuammidine, are found to be responsible for the antibacterial and antimicrobial activity of *T. procumbens* plant leaves (Ikewuchi et al., 2015).

Many studies have proved the antibacterial and antifungal potential of *T. procumben*s against human skin pathogens. According to Policegoudra et al., (2014) the methanolic extract has shown high antifungal activity against human skin pathogens like *Microsporum fulvum, M. gypseum, Trichophyton mentagrophytes, T. rubrum, Trichosporon beigelii.* Bharathi et al. (2012) reported the significant antibacterial activity of *T. procumbens* against *Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi and Escherichia coli*. According to Jain et al. (2014), methanolic extract of *T. procumbens* leaf exhibited a significant zone of inhibition against both *Fusarium oxysporum* and *Trichoderma reesei.* Kale & Dhake, (2013) investigated the antimicrobial activity of *T. procumbens* against five bacterial pathogens *S. aureus, E.*

coli, K. pneumoniae, Proteus vulgaris and *Pseudomonas aeruginosa* and reported that the methanolic extract had shown effective antimicrobial activity.

T. procumbens also exhibited anti-malarial activity when tested against chloroquine resistant *Plasmodium falciparum* using 3H – hypoxanthine assay. The results show that along with RBC protective effects the extracts of *T. procumbens* also inhibited the growth of the chloroquine resistant *P. falciparum* parasites (Appiah-Opong et al., 2011). The leaves exhibited anti-arthritic activity in complete Freud's adjuvant (CFA) induced arthritis in rats. It reduced the swelling of the rat paws and migration of leukocytes into the inflamed area (Jain et al., 2012). The aerial parts had shown hepatoprotective activity against D-galactosamine/lipopolysaccharide(D-GalN/LPS) induced hepatitis in rats. The hepatoprotective action may be mediated through the inhibition of UDP-sugar derivatives, enhancement of glycoprotein biosynthesis and stabilisation of cell membrane and inhibition of lipid accumulation by its hypolipidemic property. The hepatoprotective property of the extract may be attributed to the presence of flavonoids (Vilwanathan et al., 2005). The ethanolic extract of leaves had shown wound healing properties in streptozotocin induced diabetic and non- diabetic laboratory animals. In the excision model, animals treated with 2.5 % and 5% w/w plant extract showed significant results in wound contraction, epithelization period, and wound index (Shrivastav et al., 2020). The hydro alcoholic extract of *T. procumbens* exhibited cardioprotective activity against isoproterenol induced myocardial infarcted rats (Vadivelan et al., 2004). The acetone and ethanol extract of *T. procumbens* show anti-cancer activity on A549 (human lung cancer cell line), Hep G2 (human liver carcinoma cell line) when tested using 3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, and trypan blue dye exclusion assay method (Vishnu Priya & Srinivasa Rao, 2015). The *T. procumbens* also exhibited immunomodulatory activity (Tiwari et al., 2004), anti-hyperuricemia and antioxidant (Andriana et al., 2019). The leaves exhibited anti-inflammatory and analgesic activity when tested with formalin, acetic acid and CFA induced pain models in male C57BL6/J mice and male Sprague-Dawley rats (Prabhu et al., 2011).

Tridax procumbens leaves have good antimicrobial properties. This antimicrobial potential may help in treating the skin infections caused by certain types of microorganisms. So this study involves formulation and evaluation of herbal soap using *Tridax procumbens* leaves methanolic extract.

Methodology

Collection of Materials

The *T. procumbens* plants were collected from the Herbal Garden of Sri Krishnadevaraya University College of Pharmaceutical Sciences, Ananthapuramu, Andhra Pradesh, India. The collected plant was authenticated as *T. procumbens* (herbarium number 57417) by Botanist Prof B. Ravi Prasad Rao. The leaves were separated from the plant material. The collected leaves were washed with water and shade dried for 5 days and ground into a fine powder using a mixer grinder. The glycerin soap base is brought from Ghanshyam enterprises. Vitamin E oil is purchased from a local pharmacy. Coconut oil, Tulasi oil, and honey of different brands were purchased from the local market.

Preparation of Plant Extract

The dried leaf powder was used for the extraction of phytoconstituents. The powdered plant leaves were stored in an airtight container and the powder was extracted using methanol as solvent by the Soxhlet extraction method. *T. procumbens* dried leaf powder is weighed accurately and packed in a filter paper. The solid matrix is kept in the Soxhlet evaporator and the solvent is heated in the process of reflux. To 40 g of dried leaf powder 400 ml of methanol is used in the extraction process. Continuous extraction was done and solvent was transferred into the reservoir from the chamber. This process is continued for 8hrs and the extract is collected and concentrated using a hot water bath. Final concentrated extract is used in the formulation.

Formulation of Herbal Soap

The glassware is sterilised by dry heat sterilisation technique. 100g of glycerin soap base was weighed and melted. The glycerin soap base is prepared using coconut oil and sodium hydroxide, it is alcohol-free. In another beaker plant extract (as per formulation design), vitamin E oil and other ingredients aloe vera gel, coconut oil, honey (as per the formulation design) were mixed until all the ingredients dissolve completely. 1ml of Tulasi essential oil is added for the fragrance to the mixture. Finally, the plant extract mixture is incorporated into the melted soap base. This mixture is poured into moulds and allowed to solidify at room temperature. Four formulations were prepared. The formulation design for soaps were given in Table 1.

Phytochemical analysis of plant extract

1. Test for Flavonoids

Alkaline reagent test: Few drops of 10 % NaOH solution were added to 2 - 3 mL of extract in a test tube. Formation of intense yellow colour that becomes colourless in addition to dilute HCl indicates presence of flavonoids (Shah & Hossain, 2014).

Table 1: Formulation designs for soaps.

Ingredients	F1	F2	F3	F4
Plant extract	0.5g	1.0g	1.5g	2.0g
Soap base	100g	100g	100g	100g
Vitamin E	400mg	400mg	400mg	400mg
Tulasi oil	0.1 ml	0.1ml	0.1 ml	0.1 ml
Aloe vera gel	-	10g	-	-
Honey	5g	-	-	-
Coconut oil	-	-	-	10g
Total weight	105.9g	111.4g	101.9g	112.4g

(- means that the ingredient is not used in the formulation)

2. Test for Phenols

0.5 mL of alcoholic Ferric chloride ($FeCl_3$) solution was added to 2 mL of extract. Formation of intense bluish black colour in addition of $FeCl_3$ solution indicates presence of Phenols (Kushwaha et al., 2019).

3. Test for Tannins

Gelatin test: Gelatin solution was prepared by dissolving gelatin powder in water by heating using a water bath. To this gelatin solution 2 mL extract was added. Presence of tannins is indicated by formation of white precipitate (Kushwaha et al., 2019).

4. Test for Alkaloids

Iodine test: Few drops of dilute Iodine solution is added into 3 ml of test solution. Formation of blue colour which disappears on boiling and reappears on cooling indicates presence of alkaloids (Kushwaha et al., 2019).

5. Test for Saponins

Foam test: The extract was diluted with 20 ml of distilled water and shaken for 15 min in a graduated cylinder. Formation of foam layer indicates presence of saponins (Hossain et al., 2013).

6. Test for Steroids

2 ml acetic anhydride was added to 0.5 g of methanol extract. To this 2 ml H2SO4 was added. Colour change from violet to blue indicates presence of steroids (Kushwaha et al., 2019).

Evaluation tests

1. Examination of physical properties of formulated soap

Colour and clarity were checked by eye against white background and odour is observed. These properties were examined in all the four formulations

2. Determination of pH

5 g of soap is dissolved in 100 mL of water. The pH of the soap solution was determined using a digital pH metre (Systronics Digital pH metre MK VI). pH for four formulations was determined separately.

3. Determination of percentage free alkali

10g of sample soap was weighed using digital weighing balance (Essae Weighing balance, model- DS-852G) and taken into a beaker, 150 ml of purified water was added and boiled for 30 minutes under reflux in a water bath (SISCO water bath). The volume was made up to 250 ml in a beaker. 1ml of phenolphthalein indicator was added. It was titrated immediately with 0.1 M HCl until the solution turns colourless (Mohammed Haneefa et al., 2019).

4. Determination of foam height

0.5 g soap sample was dispersed in 25 ml purified water. It is transmitted into a 100 ml measuring cylinder and volume was made up to 50 ml with water. 25 strokes were given. It is allowed to stand till the aqueous volume is measured up to 50 ml. Foam height above the aqueous volume was measured (Ahmed et al., 2021).

5. Determination of foam retention

1% soap solution was prepared. 25 ml of 1% soap solution was taken in a 100 ml graduated measuring cylinder. The cylinder was covered and shaken 10 times. The time taken for the foam to disappear was recorded (Ahmed et al., 2021).

6. Determination of alcohol insoluble matter

Alcohol insoluble matter comprises most of the alkaline salts, such as talc, carbonates, borates, silicates and phosphates, as well as sulphates and starch, which are insoluble in alcohol under the test conditions. 5g of soap

sample was taken in a conical flask to which 50 ml of warm ethanol was added and it was shaken vigorously until the sample was dissolved completely. The solution was filtered through a tared filter paper along with 20 ml warm ethanol and dried at 1050 C for 1 hour. The weight of the dried paper was noted. (Mohammed Haneefa et al., 2019).

$$\% \textbf{ Alcohol insoluble matter} = \frac{\textbf{Weight of residue} \times \textbf{100}}{\textbf{Weight of sample}} \quad (1)$$

6. Moisture content

The moisture content is used to estimate the percentage of water present in the soap. To estimate the moisture content 5g of soap was weighed and noted as wet weight or initial weight. Using a hot air oven sample was dried at 100 to 1150C for one hour. The sample was cooled, weighed. This weight is recorded as the dry weight of the sample. Moisture content was determined using the below formula. (Mohammed Haneefa et al., 2019).

$$\% \textbf{ moisture content} = \frac{\textbf{Initial} - \textbf{Final weight}}{\textbf{Final weight} \times \textbf{100}} \quad (2)$$

Results

The formulated soaps were examined for physical parameters like colour, clarity, and odour. Evaluation parameters like pH, percentage free alkali, foam height, foam retention, and alcohol insoluble matter, Percentage of moisture content. The results for phytochemical analysis were given in Table 2, while the results of evaluation studies for the four formulations F1, F2, F3, F4 are given in Table 3.

Table 2: Phytochemical analysis of *T. procumbens*

Phytochemical analysis	Observation	Results
Test for Flavonoids (Alkaline reagent test)	Dark yellow colour was formed	+
Test for Phenol	Bluish black colour was observed	+
Test for Tannins (Gelatin Test)	White precipitate was formed	+
Test for Alkaloids (Iodine test)	Blue colour is observed and disappeared on heating	+
Test for Saponins (Foam test)	Foam was generated on shaking	+

Table 3: Evaluation parameters for formulations

Evaluation parameters	F1	F2	F3	F4
Colour	Dark green #006400	Dark green #006400	Dark green #006400	Dark green #006400
Clarity	Good	Good	Good	Good
Odour	Tulasi odour	Tulasi odour	Tulasi odour	Tulasi odour
pH	8.9	8.7	9.3	8.19
Percentage free alkali	0.68%	1.28%	1%	0.88%
Foam height	35ml	36ml	53ml	5ml
Foam retention time	13 min	100min	110 min	33 min
% alcohol insoluble matter	4%	16%	9.5%	10%
Moisture content	18%	28.4%	18.8%	23%

Discussion

The methanolic extract of *T. procumbens* consists of saponins, alkaloids, tannins, phenols, flavonoids and steroids. All the soaps have a dark green colour, good clarity, and good odour. All the soaps have pH in the range of 8-9.1. So the soaps are a little basic in nature. Percentage free alkali refers to the free or excess unreacted base (alkali) present in the formulation. These free bases cause irritation on human skin. Among four soaps F2 has a high percentage of free alkali, F1, F3 and F4 are within the range of 0.6 - 1. F1 soap has the least percentage of free alkali i.e, 0.68% when compared to other formulations. Matter insoluble in alcohol is a parameter used to determine the purity of soap (Vivian et al., 2014). According to the Bureau of Indian standards, 10% of

alcohol insoluble matter is the limit for toilet soaps of grade 2 and grade 3 (IS 2888: 2004). F1, F3, F4 have 4%, 9.5%, 10% respectively. Among all four soaps F1 has the least amount of alcohol insoluble matter i,e 4 % indicating it has the least number of impurities in it than other formulations. Moisture content indicates the presence of moisture in the sample; if it is high the formulation will get deteriorated easily. High moisture content means it releases water, which reacts with unsaponified fat in the soap and causes hydrolysis of soap releasing free fatty acids and glycerol (Vivian et al., 2014). Among the four soaps F1, F3 have low moisture content compared to F2 and F4. Foam height and foam retention time determine the foaming activity and cleaning efficacy of the soap. Among the four formulations F1, F2, F3 showed good foam heights in the range of 35 to 53 ml. All four soaps had a relatively long period of foam retention. F3 soap has foam for a longer period i.e, 110 minutes. All the four soaps showed stable foam for more than 5 minutes. All four samples had shown good foaming properties.

Conclusion

In this present study, four herbal soaps were prepared using *Tridax procumbens* leaves extract with varying quantities of ingredients and drug. All the formulated soaps had good appearance, uniform colour, good odour. F1 has the least content of free alkali (0.68%). As free bases in soap cause irritation, these should be as low as possible. When compared to other soaps F1 has least making it good when compared to other soaps. It has least amount of matter insoluble in alcohol (4%), which is very less compared to other formulations and it is also in the acceptable limit (10%) of BIS. The moisture content of the F1 soap is also less compared to other soaps, which is another parameter, which makes it better than others. F1 had shown stable foam for more than 5 min. F3 has shown stable foam than other formulations. But based on these considerations it can be concluded that F1 soap had shown better results than other soaps. Naturally *Tridax procumbens* leaves have good antimicrobial properties. The other ingredients like coconut oil, aloe vera gel, Vitamin E oil, Tulasi oil used were proven to be dermatologically safe and helps in providing additional benefits to skin like moisturising effect, and conditions skin. So, the potential use of the formulated soaps in treating skin infections can further be explored. Herbal drugs like *T. procumbens* can be formulated in the form of soaps.

But F1 had shown the best results among all for evaluation studies. It has good foaming property and the least number of impurities as per the standards of the Bureau of Indian Standards. The potential use of the formulated soaps in treating skin infections can further be explored. Herbal drugs like *T. procumbens* can be formulated in the form of toilet soaps.

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Conflict of Interest

The authors declare no conflict of interest.

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