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Formulation and evaluation of medicated polyherbal tattoo ink

Raslamol K *, Amrutha Chandran, Anita Thomas, Delsha Davis, Mariya M. P and Silpa Kumar

Department of Pharmaceutics, Nirmala college of Health Science, Meloor, Chalakkudi, India.

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Abstract

Transdermal tattoos offer a potential avenue for administering drugs externally into the dermis, resulting in the creation of a lasting mark. Analyzing complex samples is crucial to assess the presence of ingredients, ensuring the safety of cosmetic products that contribute to health protection. This investigation aims to formulate and assess tattoo ink for treating allergies and inflammation. The study proposes a comprehensive analytical approach, encompassing physical appearance, homogeneity, pH, spreadability, viscosity, and antibacterial evaluation to characterize samples of henna and annatto. Standardizing herbal formulations is imperative to evaluate drug quality based on the concentration of active principles. This paper focuses on the standardization of Henna (*Lawsonia inermis* Linn) from the Lythraceae family and Annatto from the plant Bixa Orellana. Annatto, known for its lower toxicity and improved biodegradability, can be extracted using water or organic solvents to obtain pigment, tocotrienol, and geranylgeraniol components. These compounds exhibit antibacterial activity, inhibit certain types of cancer, and demonstrate hypocholesterolemic effects, among other properties.

Keywords: Henna; Annatto; Transdermal drug delivery; Antibacterial activity; Standardization.

1 Introduction

A medical tattoo serves various purposes, including treating a condition, conveying information, or marking a specific body location. The term originates from the Tahitian word 'tatu,' signifying the act of marking for symbolic representation or identification.

Medicated tattoos, also known as Med-Tats, represent an emerging approach in transdermal drug delivery. Unlike conventional tattoos used for amusement, medicated tattoos contain active medicinal ingredients. Pharmaceutical companies carefully select substances, such as alcohol, to enhance skin penetration within the patch, thereby improving absorption. Prototype Med-Tats typically feature acetaminophen and vitamin C as drug candidates. Tattoos also serve a critical role in alerting emergency personnel to a person's diabetes mellitus, a condition where individuals may enter a diabetic coma, rendering them unable to communicate. In reconstructive surgeries like breast reconstruction after mastectomy or breast reduction, tattooing is employed to replace the removed areola or fill in areas of pigment loss. Some hospitals even offer free nipple tattoos to breast surgery patients for scar camouflage.

Vinnie Myers of Little Vinnie's Tattoos in Finksburg, Maryland, has tattooed over 5,000 women who underwent breast cancer surgery, offering a significant service in scar camouflage. Similarly, a cosmetic tattooist in the UK provided a similar service without charge, with bookings made six months in advance. Another post-mastectomy option is opting for a decorative chest tattoo as a form of body art rather than reconstruction.

Beyond medical applications, tattoos find use in simulating the appearance of fingernails and concealing scars. Additionally, micro-pigmentation, or permanent makeup, can be utilized to minimize the visibility of vitiligo-affected skin areas.

* Corresponding author: Raslamol K

2 Ink used in tattoo

Tattoo supply stores offer specialized inks designed for creating tattoos, available in various colors and typically packaged in 4 oz. plastic squeeze bottles for easy dispensing. These liquid pigment dispersions are approved by the Food and Drug Administration in the United States, consisting of dyes derived from metal components. However, allergic reactions to this type of ink are possible due to its composition. In contrast, vegetable inks, based on organic products, are considered advantageous as they are less likely to cause adverse skin reactions. Despite rumors suggesting that designs may fade more easily over time, these inks boast better assimilation and absorption by the body compared to acrylic inks. Notably, vegetable inks are vegan, containing no animal ingredients. While the advantages of each ink type are enticing, it is crucial to be well-informed to make conscious choices, considering the desired result and potential physical reactions.

For individuals with chronic allergies, a medical consultation is advisable. Some vegan and non-toxic ingredients found in different ink colors include carbon and logwood for black, titanium dioxide for white, turmeric for yellow, and monoazo for green. Sodium and aluminum contribute to blue ink.

Advantages of medicated tattoos include their ability to deliver drugs to children who cannot tolerate conventional dosage forms. These tattoos are attractive, easy to apply, enhance skin appearance, and may nourish the skin with added nutrients and vitamins for expedited healing. However, tattoos pose disadvantages and associated risks, such as potential cancer-causing agents, the risk of Hepatitis due to needle use, and pain during the needle penetration process. Applications of medicated tattoos extend to children who fear injections, patients undergoing breast reconstruction surgery, women covering scars after breast surgery, medical research for DNA vaccination strategies, and indicating disease states like diabetes, epilepsy, and heart strokes. Henna, derived from the dried leaves of the henna shrub (*Lawsonia inermis*), is a red or brown dye commonly used for temporary tattoos, especially on hands and feet. The combination of chemicals and nutrients in henna imparts anti-inflammatory, antibacterial, astringent, and antiviral qualities.

3 Plant profile

3.1 Bixa Orellana

Figure 1 Seeds of Bixa Orellana

Kingdom: Plantae , Clade: Tracheophytes , Clade: Angiosperms , Clade: Eudicots ,Clade: Rosids ,Order: Malvales ,Family: Bixaceae ,Genus: Bixa ,Species: B. Orellana

3.1.1 Uses

As a plant high in antioxidants, annatto likely has benefits that might reduce the expansion and spread of cancer tumors. Annatto also has properties that will calm indigestion, relieve heartburn and ease the pain of stomach cramps. For stomach-pain relief, add annatto to herbal tea or other warm beverages. Annatto is additionally a coloring agent made up of the seeds of the plant Bixa Orellana, commonly called the lipstick tree. The cosmetic industry has used annatto in the formulation of lipsticks, shampoos, soaps and other skincare products.

3.2 Lawsonia Inermis

Kingdom: Plantae , Clade: Tracheophytes , Order: Myrtales , Family: Lythraceae , Subfamily : Lythroideae , Genus: Lawsonia , Species: L. inermis

Uses

Regulated Blood Pressure, Dysentery, Anti-aging Properties, Improved Nail Quality, Baldness, Arthritis, Headache Reliever, Wound Healing, Cure Fever, Anti-inflammatory Capacity, Reduced Sleep Issues, Hair Color, Detoxification.

Figure 2 Leaves of *Lawsonia Inermis*

4 Material and methods

4.1 Henna

Collection and Authentification of the plant *Lawsonia* inerms (SPECIMEN NO- 178244) was been done by A.K.Pradeep sir dept of Botany of Calicut University, Kozhikode.

4.1.1 Methodology

- The fresh leaves of henna plant was taken and then dried under shade and then powdered.
- Henna powder is been taken in the beaker and to it a cup of water is been added and produced to flame
- The beaker is then stirred continuously , the boiled mixture was set for a period of time and filter the contents.

Figure 3 Extraction of Henna leaves

4.2 Annatto

Collection and Authentification of the plant Bixa orellena (SPECIMEN NO- 178243) was been done by A.K.Pradeep sir dept of Botany of Calicut University, Kozhikode

4.2.1 Methodology

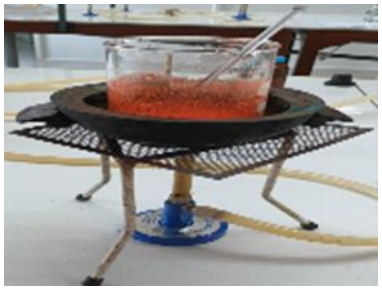

Figure 4 Extraction of annatto seed

- The Annatto seeds are been collected from the plant and the. Dried under shade and then removed from pod.
- The seeds are then powdered and then kept aside.
- To the powder a pinch of common salt is added and then the ¾ water is added to it .
- Then vegetable oil is been added to it and then mixed thoroughly to get the ink

4.2.2 Formulation of tattoo ink

- The 2 ml of Henna extract and 2 ml of Annatto extract are taken and transfer into a beaker.
- 2 ml of ethyl alcohol is been added to it and stirred well.
- To the above mixture 1 ml of propylene glycol and 1 ml of chloroform.
- 0.5 ml of Peppermint oil,1 ml of glycerine and 1 ml of gelatin is added.
- 1 ml of Sodium benzoate solution is added as preservative.

4.3 *Physicochemical evaluation*

4.3.1 Determination of extractive value

This method determines the number of active constituent in each amount of plant material when extracted with the solvent. The extractive value used as a means of evaluating crude drug which are not readily estimated by other means.

% EXTRACTIVE VALUE =WEIGHTOF EXTRACT/WEIGHT OF SAMPLE *100

4.3.2 Determination Of Alcohol Soluble Extractive Value

Weigh about 5 gram powdered drug with 100 ml of alcohol in a stoppered flask for 24 hours and shaking for 6 hours. Filter rapidly through filter paper. Taking precaution against excessive loss of alcohol. Evaporate 25 ml of alcoholic extracted to dryness in a tarred flat bottomed dish (32). Dry at 800C and weigh. Keep it in a desiccator.

Calculate the percentage w/w of alcohol soluble extractive with the reference to the air dried drug.

4.3.3 Determination of Water Soluble Extractive Value

Determination of water soluble extractive value follows the procedure as above using chloroform. Water is used instead of alcohol.

4.3.4 Determination of Ash Value

Ash value is helpful in determining the quality and purity of a crude drug. On incineration crude drug normally leaves an ash usually consisting of carbonate, phosphate and silicate of sodium, potassium, calcium and magnesium. Weigh about 3 gram of the powdered drug in a tarred silica crucible and incinerate the powdered drug by gradually increasing the temperature until free from carbon and cool it. Keep it in a dessicators (37). Weigh the ash and calculate the percentage of total ash with reference to the added dried sample.

TOTAL ASH =WEIGHT OF ASH /INITIAL WEIGHT*100

4.3.5 Determination Of Moisture Content

The principle of determinating the thermogravimetric method of moisture content is defined as the weight loss of mass that occurs when the material is heated. The sample weight is taken prior to heating and again after reaching a steady-state mass subsequent to drying (15). Presence of moisture content in a crude drug can lead to its deterioration due to the either activation of certain enzyme or growth of microbes.

Moisture content can be determined by heating the drug at 60 0C in a oven to a constant weight and calculating the loss of weight.

Place 10 gram of drug in a tarred evaporating dish. For unpowered part the sample was prepared by cutting. Placing the drug in a tarred evaporating dish dry at 105°C for 5 hour(7). Then weigh it and continue the drying and weighing at one hour interval until the difference between two successive weight corresponding to not more than 0.25%. constant weight is reached when two consecutive weight after drying for 30 minutes and cooling for 30 minutes in a desiccator.

Weight of weighing bottle= W1, Weight of bottle + drug= W2, Weight of drug =W2-W1

Final weight of the drug =W4

MOISTURE CONTENT =W3 -W4/W3 ×100

Table 1 Phytochemical Screening

Phytoconstituents	Test	Observation
Alkaloids	MAYER'STEST- 2 ml extract +few drops of mayer's reagent	Yellow precipitate
Flavanoids	LEAD ACETATE TEST- Add few drops of aqeous basic lead acetate solution to 1 ml of alcoholic extract using a test tube.	Appearance of a reddish brown bulky precipitate.
Carbohydrate	MOLISCH TEST-2 ml extract=10 ml water=2drop of ethanolic alpha naphthol(20%)+2 ml conc sulphuric acid.	Reddish violet coloured ring at the junction.
Glycoside	Keller kiliani test- Add 1 ml of extract+glacial acetic acid +1 ml of Ferrous suphate +1 ml conc Sulphuric acid.	Blue color
Tannins	Ferric chloride test- 2 ml extract + 1 ml of Ferric chloride	Blackish blue precipitate
Steroid	Libermann Burchad test-2 ml extract +1 ml of acetic anhydride+2 ml of ethanolic extract+conc Sulphuric acid	Green color
Protein	Ninhydrin test-1 ml extract+2 ml ninhydin reagent	Violet precipitate
Saponin	Foam test- 5 ml extract+5 ml water+heat	Froth appears
Phenols	Ferric chloride test-extract was treated with 3-4 drops of ferric chloride.	Formulation of bluish black color.

5 Formulation

- Phase A: Propylene glycol, chloroform, peppermint oil, glycerine are stirred together until a uniform mixture is formed.
- Phase B: Purified water is added to phase A.

To the above mixture the contents of Phase –C add the extracts of *Curcuma longa, Bixa orellena ,Lawsonia inermis* separately at varying concentration with continuous stirring. Finally the above made different formulations are been added to the Phase –D containing sodium benzoate . Twelve different formulation were prepared.

These preparations are denoted as F1,F2,F3,F4,F5,F6 for alcohol and F7,F8,F9,F10,F11and F12 for aqueous extract.

Table 2 Formulation of tattoo ink using Henna extract and Anatto

Composition of tattoo ink	F1 %w/w	F2 %w/w	F3 %w/w	F4 %w/w	F5 %w/w
Henna extract	1	2	3	4	5
Anatto	1	2	3	4	5
Propylene glycol	0.5	0.5	0.5	0.5	0.5
Glycerine	0.5	0.5	0.5	0.5	0.5

Sodium benzoate	0.5	0.5	0.5	0.5	0.5
Peppermint oil	0.5	0.5	0.5	0.5	0.5
Gelatin	0.5	0.5	0.5	0.5	0.5
Chloroform	0.5	0.5	0.5	0.5	0.5
Distilled water q.s	1	1	1	1	1

5.1 Evaluation test

5.1.1 Physical Appearance

The physical appearance, color, and feel of the prepared poly herbal tattoo ink was visually tested.

5.1.2 Homogeneity Test

A clean and dry object glass was smeared with the tattoo ink, and a cover glass was sealed. The appearance under the light of some coarse particle/homogeneity was investigated (22, 35). Herbal tattoo ink was tested by visual examination for homogeneity and tested for some lumps, flocculates, or aggregates

5.1.3 PH Test

The pH test will be determined by using Digital pH meter. Dipper of digital pH will be dip into the sample of serum formulation and the pH value will be recorded. The pH meter was calibrated using pH 4 and pH 7 buffer solutions (43). Then, the electrode was soaked in the tattoo ink and left until the pH normalized after a few minutes.

5.1.4 Viscosity Test

The viscosity measurement was performed with Ostwald viscometer by measuring the time for a known volume of the liquid (the volume contained between the marks A and B) to flow through the capillary under the influence of gravity.

5.1.5 Spreadability Test

The product's coverage on the skin or affected area indicates the extent to which the tattoo ink was applied. Various sizes of filter paper are selected, and each piece is assessed by measuring the total area (A1) and weighing (W1). To conduct the test, choose the formulation for evaluation and draw several milliliters into a B-D 5 ml syringe, applying it to the center of the filter paper in 20 drops (17). Initiate a timer when the last drop hits the filter paper, precisely counting down for 10 minutes. Throughout this period, the liquid will unifor mly spread in a circular pattern over the filter paper.

After the 10-minute duration, use scissors to cut precisely along the line between the saturated spread and the dry filter paper. Weigh the remaining dry filter paper and record this weight as W2. Measure the diameter of the saturated portion of the filter paper (17.8). In cases where the spread is not a perfect circle, take several diameter readings around the spread area and calculate an average diameter. Document this measurement as A2.

$$\% \text{ Spread by Area} = (A2/A1)100$$

5.1.6 FTIR (Fourier Transform Infrared Spectroscopy)

Principle: In infrared (IR) spectroscopy, the sample allows the passage of IR radiation, absorbing a portion of it. The transmitted radiation through the sample is then recorded.

Significance: Fourier-transform infrared spectroscopy (FTIR) is the favored technique for infrared spectroscopy for various reasons:

- It does not cause destruction to the sample.
- It is considerably faster compared to older methods.
- It exhibits higher sensitivity and precision.

5.1.7 Antibacterial

Antibacterial activity can be assessed and evaluated by examining the bacterial species' growth in the culture medium using the agar plate diffusion method. The formation of an inhibition zone is observed, and the antibacterial efficacy is assessed against a chosen standard. This is then compared with a test compound known for its antibacterial properties.

6 Result of evaluation

6.1 Physical Appearance

The physical appearance, color, and feel of the prepared herbal tattoo ink are visually tested. Tattoo ink formulation was green viscous liquid preparation with a smooth homogeneous texture and glossy appearance . Consistency was found to be good. Consistency and appearance was found to be appreciable in formulation F5.

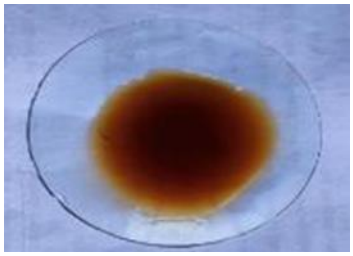

Figure 5 Physical Appearance

6.2 Homogeneity Test

Figure 6 Homogenicity Test

By visual examination of the appearance and presence of any lumps, flocculates, or aggregates, in the produced herbal tattoo ink was checked for homogeneity. The homogeneity of prepared ink has been shown to be fine**.**

6.3 pH TEST

The pH of the whole herbal tattoo ink was found to be in the range of 3.5-5.5 which was sufficient for the skin, suggesting that the herbal tattoo ink was suitable for the skin.

Figure 7 pH test

6.4 Viscosity Test

The viscosity measurement was performed and recorded using Ostwald viscometer. The viscosity was recorded for five different concentrations formulations .

Figure 8 Viscosity Test

6.5 Spreadability Test

From the result of evaluation, it is showed that formulation 1 given the higher percentage of spreadability with 24% compared to other formulations(5,7). Secondly, the formulation 2and 3 also showed more than 20% percent of spreadability during the test carried out while formulation 4and 5 only give 16.8% and 16% of spreadability respectively and formulation F6 with 17.5% spreadability.

Figure 9 Spreadability

6.6 Redispersion test

Redispersion test carried out using the formulation was redispersed within seconds after doing their dispersion test micro centrifuge each formulation gave satisfactory redispersion results upon redispersion.

Figure 10 Redispersion Test

7 Conclusions

Medicated tattoos, as a transdermal drug delivery system, have garnered recognition despite inherent limitations. This system offers controlled-rate delivery, minimizing side effects while ensuring enhanced efficacy and constant drug release. Human skin, known for its formidable barrier function, often necessitates the application of enhancement strategies to facilitate the penetration of active ingredients.

The field of medical science has witnessed a significant surge in the popularity of tattooing. Particularly, there is a growing application of tattoos in medical identification, catering to conditions that demand special attention during emergencies. For instance, tattoos serve as a crucial identifier for patients with diabetes, aiding in situations where unconsciousness may result from hypoglycemia or an allergy to a specific medication.

The current discourse delves into well-described treatment options for tattoos, emphasizing the need for individualized approaches aligned with patient safety concerns. Tattoos prove to be reliable and efficacious tools for diverse purposes, including amateur, professional, cosmetic, and traumatic applications. Furthermore, the incorporation of nontoxic and nonirritant biodegradable herbal pigments in medicated tattoos holds the potential to revolutionize the field of medical science.

Medicated tattoos are essentially modified temporary tattoos containing active drug medicaments for transdermal delivery. Their application is both attractive and enjoyable, involving wetting with water and pressing onto the skin. The tattoo comprises a drug layer, a colored design layer, and an adhesive layer binding to the skin. The duration of therapy is not predetermined, and manufacturers provide a color chart for comparison, indicating when the tattoo should be removed.

Offering a visual indication of drug absorption into the skin, the tattoo gradually fades away painlessly, and removal is simple through an astringent wash containing isopropyl alcohol. Prototype medicated tattoos may include drugs such as acetaminophen and vitamins.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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