

TO FORMULATE AND EVALUATE HERBAL BURN AND WOUND HEALING OIL FOR DIABETIC PATIENTS

Pratiksha D. Gadekar¹, Rutuja B. Bhand², Bhavesh V. Kapadi³, Ajay S. Kalekar⁴,
Aniket D. Avhad⁵, Pankaj V. Gund*⁶, Prasad A. Salve⁷, Vaibhav T. Shete⁸, Yashanjali
R. Abhale⁹ and Minaj B. Inamdar¹⁰

^{1,2,3,4,5,6,7,8,9}Students, Samarth College of Pharmacy, Belhe, Pune, Maharashtra, India.

¹⁰Assistant Professor, Samarth College of Pharmacy, Belhe, Pune, Maharashtra, India.

Article Received on
25 April 2024,

Revised on 15 May 2024,
Accepted on 04 June 2024

DOI: 10.20959/wjpr202412-32580



*Corresponding Author

Pankaj V. Gund

Students, Samarth College
of Pharmacy, Belhe, Pune,
Maharashtra, India.

ABSTRACT

To formulate and evaluate herbal burn and wound healing oil for diabetic patient. Burn and wound healing is a complex process in the biological objectives and mechanisms defined by the initial injury. The majority of the peoples prefer natural products for their personal care rather than chemical products because natural products are free from harmful chemicals, improves the health of the skin, and provides the nutrients, satisfaction for users. The purpose of the present work was to formulate and evaluate herbal burn and wound healing oil for diabetic patients. In the present work herbal burn and wound healing oil were prepared by using magnetic stirrer. The prepared oil formulation were evaluated for pH, viscosity, spreadability, stability, drug content, etc.

KEYWORDS: Burn healing, wound healing, diabetics, Burn injury.

INTRODUCTION

One of the most health-threatening problems in the world is burn injury. Over 6.6 million people worldwide suffer from burns, and almost 256,000 of them die annually. About 1% of all deaths are related to burn injuries. A burn is a skin injury that can lead to tissue dysfunction. Thermal, chemical, and electrical burns are the most common types of burn injuries. Skin with about 15 % of body weight is the largest organ of the integumentary system and has several essential functions. The most important is the prevention of organism desiccation and the protection of inner body structures from the environment. The skin has a multilayer structure and any component has specific properties.^[1]

Burn injury is first and foremost an injury to the skin.^[2] Burns remain as one of the most common injuries worldwide, with more than one million patients annually in the USA alone.^[3] Although the burned patient has many problems to face during the stages of recovery from a burn injury the major and persisting problems for survivors, the vast majority of patients, are those associated with the problems of healing and the final outcome of healing in terms of scarring.^[2] A burn ensues after the skin is damaged by heat, radiation, electricity, or chemicals. Serious complications of deep or widespread burns can happen, e.g., sepsis due to bacterial infection, shock caused by hypovolemia, or scarring tissue contraction after improper wound healing. Thermal burn and related injuries have remained a major cause of death and disability.^[3]

‘Healing’ has many meanings. In its broadest context it can be used to describe the restoration of normality to any fractured situation. In its context as applied to wounds it should be taken to mean any process which acts, or is induced to act, to restore the integrity of the damaged tissues with replacement of lost tissues and repair of damaged tissues. In the context of clinical practice in wound treatment ‘healing’ could be taken to have two main connotations. The first represents the innate properties of the systems of the body which act to replace and repair damaged tissues. The second is the intervention of a third party who carries out an active process designed to accelerate or modify the natural process of healing for the benefit of the patient. This third party intervention is conventionally understood as the surgical intervention necessary to produce the conditions that will lead to the spontaneous healing of the wound or the application of biological or biocompatible materials which act to close the wound and reach a stable acceptable end point to the healing process.^[4]

According to the depth, burn wounds are classified as first degree (superficial), second degree (partial thickness) and third degree (full thickness). In this study, we focus on discussing the essentials, achievements, and challenges of cell-based therapy for skin tissue regeneration in the treatment of burn injury.^[5]

Wound is defined as disruption of cellular, anatomical, and functional continuity of a living tissue. It may be produced by physical, chemical, thermal, microbial, or immunological insult to the tissue. Wound healing is the interaction of a complex cascade of cellular and biochemical actions leading to the restoration of structural and functional integrity with regain of strength of injured tissues. The wound healing is a multi-stage phenomenon that requires the activation, induction of numerous cell types as keratinocytes, endothelial cells,

fibroblast, inflammatory cells, and macrophage which appear to be central to this process. Wound healing is a complex process, divided into at least three phases: Inflammatory phase, proliferative phase, and remodeling phase.^[6]

MATERIAL AND METHODS

A] Material

1. Turmeric oil were purchased from Seyal Natural 16-1-27/C/28 Jaya Nagar Saidadad, Hyderabad Telengana 500059.
2. Aloe vera oil were purchased from deve herbes UG-12 Westend Mall Janakpuri, New Delhi.
3. Tea tree oil were purchased from Manchanda Herbal Product E-64 (B.S) and F-78 Indl Area Haridwar 249401 (U.K).
4. Sandalwood oil were purchased from A Quality product from the house of Sahil Exclusive. Lemongrass oil were purchased from AG Industries Noida.
5. Methanol (LR Grade) were purchased from GS LAB.
6. Ethanol (LR Grade) were purchased from Thermosil fine chemicals.
7. Dichloromethane were purchased from Thermosil fine chemicals.
8. Potassium hydroxide were purchased from Thermosil fine chemicals.

B] Extraction Method

Extraction was carried out by Clevenger-type apparatus (Ashk-e shisheh Co., Iran) in accordance with the description of the British Pharmacopoeia for 3(British Pharmacopoeia 1999). Hundred gram of powdered rhizome were mixed with 1,000 mL of distilled water into the 2,000 mL balloon. About 1.8 mL oil, present at the upper layer in the tube, was separated from the water. The essential oil was stored in a dark glass bottle at 4C before the examination of the presence of curcumin.^[7]



Figure 1: Extraction method by Clevenger Apparatus.

Preparation of burn and wound healing oil

The oil formulation was prepared by simple solution method. Briefly, the oil was mixed by constant stirring using magnetic stirrer. The oil was allowed to mixed for 2 hours placed on digital magnetic stirrer maintained at speed 300 rpm.^[8,9]



Figure 2: Magnetic stirrer.

EVALUTION PARAMETERS

A] Evaluation Parameters of Oils

Standardization of oil

The standardization of all oils was carried out as per standard procedure for UV analysis, acid value, peroxide value, saponification value, iodine value, ester value, skin irritation test, pH, appearance, stability, viscosity, specific gravity.

I] Uv Analysis

Method Development

Instruments - Spectroscopic analysis was carried out using Shimadzu UV-Visible Spectrophotometer.

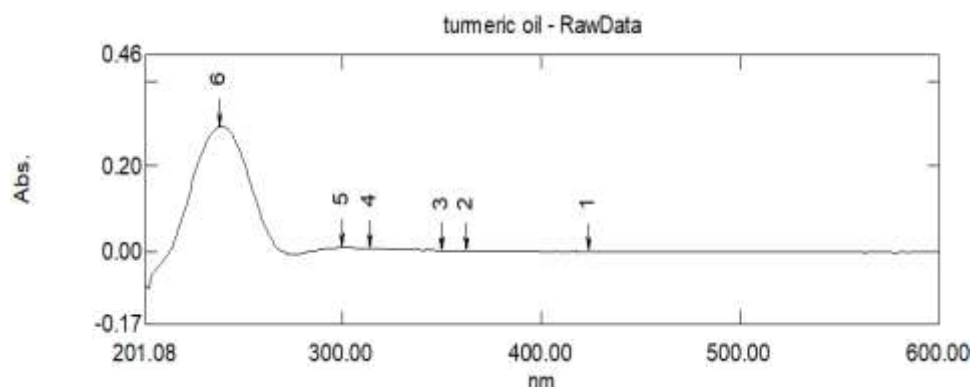
A] Turmeric oil

Preparation of standard solution of Turmeric oil

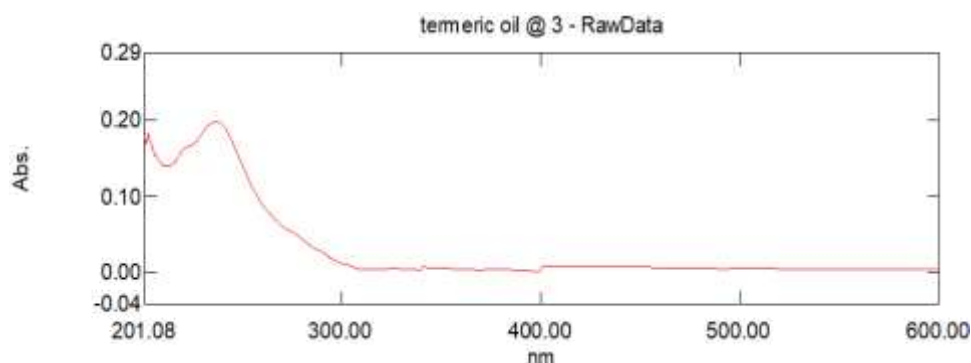
Turmeric oil 1 ml was accurately weighed and transferred in a 10 ml volumetric flask. Methanol was added up to the mark to obtain a concentration of 10 μ g/ml of Stock solution.

UV spectrum of Turmeric oil standard solution

Standard solution of Turmeric oil was used for a spectral scan of Turmeric oil between 400-800nm using the solvent system as blank.^[10,11]



Graph 1: Uv spectrum of extracted turmeric oil.



Graph 2: Uv spectrum of turmeric oil.

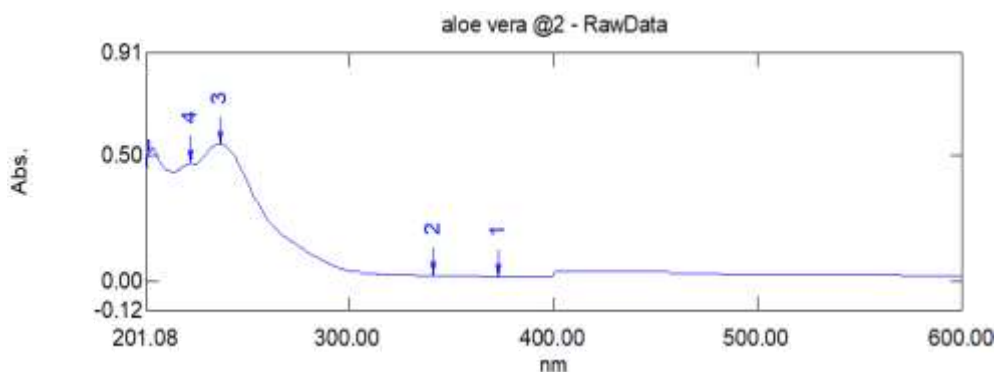
B] Aloe vera oil

Preparation of standard solution of Aloe vera oil

Aloe vera oil 1 ml was accurately weighed and transferred in a 10 ml volumetric flask. Methanol was added up to the mark to obtain a concentration of 10 μ g/ml of Stock solution.

UV spectrum of Aloe vera oil standard solution

Standard solution of Aloe vera oil was used for a spectral scan of Aloe vera oil between 200-400nm using the solvent system as blank.^[12]



Graph 3: Uv spectrum of aloe vera oil.

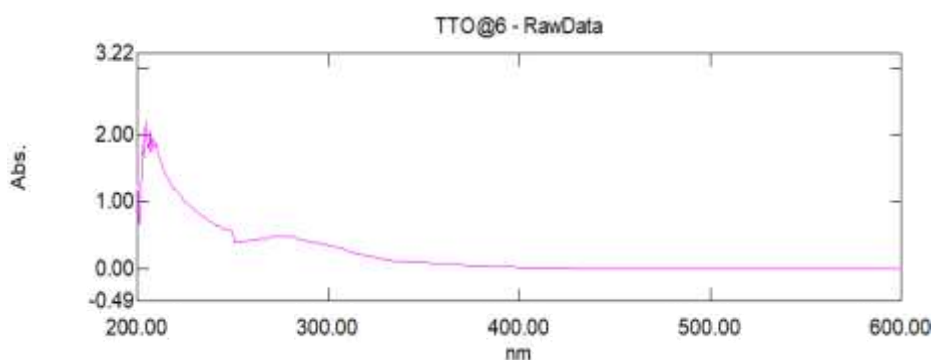
C] Tea Tree Oil

Preparation of standard solution of TTO

1 ml TTO was weighed accurately into a 10ml volumetric flask, dissolved using DCM-Me OH (3:2v/v) solvent system and volume made up to the mark using the same. The concentration of the resulting solution was 10mg/ml of TTO.

UV spectrum of TTO standard solution

Standard solution of TTO was used for a spectral scan of TTO between 400-250nm using the solvent system as blank.^[13,14]



Graph 4: Uv spectrum of tea tree oil.

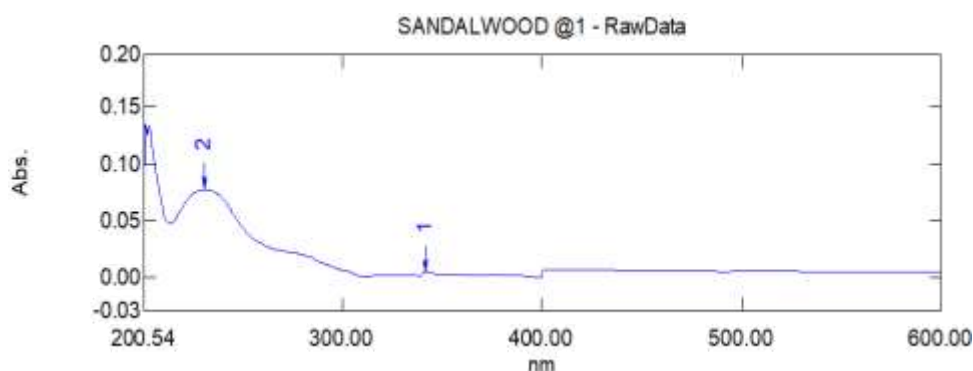
D] Sandalwood oil

Preparation of standard solution of sandal wood oil

Sandalwood oil 1 ml was accurately weighed and transferred in a 10 ml volumetric flask. ethanol was added up to the mark to obtain a concentration of 10µg/ml of Stock solution.

UV spectrum of sandalwood oil standard solution

Standard solution of sandalwood oil was used for a spectral scan of sandalwood oil between 400-800nm using the solvent system as blank.^[15]



Graph 5: Uv spectrum of sandalwood oil.

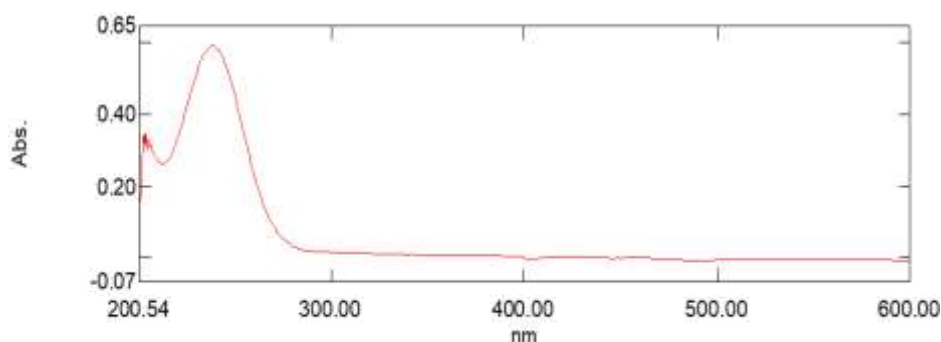
E] Lemongrass oil

Preparation of standard solution of Lemongrass oil

Lemongrass oil 1 ml was accurately weighed and transferred in a 10 ml volumetric flask. Methanol was added up to the mark to obtain a concentration of 10 μ g/ml of Stock solution.

UV spectrum of Lemongrass oil standard solution

Standard solution of Lemongrass oil was used for a spectral scan of Lemongrass oil between 200-400nm using the solvent system as blank.^[16]



Graph 6: Uv spectrum of lemongrass oil.

Table 1: oils and their absorbance.

Sr. no	Oils	λ_{max}	Solubility
1	Turmeric oil	425 nm	Methanol
2	Aloe vera oil	267 nm	Methanol
3	Lemongrass oil	264 nm	Methanol
4	Sandalwood oil	412 nm	Ethanol
5	Tea Tree oil	267 nm	Dichloromethane in methanol

II] Acid value

The acid value (AV) is the number that expresses, in milligrams the quantity of potassium hydroxide (KOH) required to neutralize the free acids present in 1 g of the substance.

Significance

Acid value is the measure of hydrolytic rancidity. In general, it gives an indication about edibility of the lipid.

- Edible oil contain > 1%
- Pharmaceutical oil must not have any acidity.

Formula

$$\text{Acid value} = 5.61 \times \frac{n}{W}$$

Where,

n= The no of ml of 0.1M KOH required

W= The weight in gm of the substance

Procedure

Take 1 gm of Oil Sample in Conical Flask.



Add 5 ml of Ethanol in the conical Flask.



Add 2-3 drop of Phenolphthalein Indicator.



Titrate with 0.1 N of KOH solution.^[17]



Figure 3: Titration of Acid Value.

Table 2: oils and their acid value.

Oils	Acid value
Turmeric oil	2.805
Tea tree oil	6.732
Aloe vera oil	4.488
Sandalwood oil	9.537
Lemongrass oil	8.415

III] Saponification value

The saponification value is the number of mg of potassium hydroxide required to neutralize the free acids and to saponify the esters in 1 g of the substance.

Method

Unless otherwise specified in the individual monograph, introduce about 2 g of the substance under examination, accurately weighed, into a 200-ml flask of borosilicate glass fitted with a reflux condenser. Add 25.0 ml of 0.5 Methanolic potassium hydroxide and a little pumice powder and boil under reflux on a water-bath for 30 minutes. Add 1 ml of phenol- phthalein solution and titrate immediately with 0.5 M hydrochloric acid (ml). Carry out a blank titration omitting the substance under examination (b ml). Calculate the saponification value from the expression

Formula

$$\text{Saponification value} = 28.05 \times \frac{(b-a)}{W}$$

W = weight in g of the substance.^[17,18]

IV] Iodine value

The iodine value is the number which expresses in grams the quantity of halogen, calculated as iodine, which is absorbed by 100 g of the substance under the described conditions. It may be determined by any of the following methods.

Method A (Iodine Monochloride Method or Wijs Method)

Place an accurately weighed quantity of the substance under examination in a dry 500-ml iodine flask, add 10 ml of carbon tetrachloride and dissolve. Add 20 ml of iodine monochloride solution, insert the stopper and allow to stand in the dark at a temperature between] 5° and 25° for 30 minutes. Place 15 ml of potassium iodide solution in the cup top, carefully remove the stopper, rinse the stopper and the sides of the flask with 100 ml of

water, shake and titrate with 0.1 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator.

Note the number of ml required.

(a) Repeat the operation without the substance under examination and note the number of ml required.

(b) Calculate the iodine value from the expression.

$$\text{Iodine value} = 1.269 \frac{(b-a)}{w}$$

Where,

w = weight in gm of the substance.

The approximate weight in gm of the substance to be taken may be calculated by dividing 20 by the highest expected iodine value. If more than half the available halogen is absorbed, the test must be repeated with a smaller quantity of the substance.^[18]

V] Peroxide value

The peroxide value is the number of milliequivalents of active oxygen that expresses the amount of peroxide contained in 1000 g of the substance.

Method

Unless otherwise specified in the individual monograph, weigh accurately about 5 g of the substance under examination, transfer to a 250-ml glass-stoppered conical flask, add 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform, swirl until dissolved and add 0.5 ml of saturated potassium iodide solution. Allow to stand for exactly 1 minute, with occasional shaking, add 30 ml of water and titrate gradually, with continuous and vigorous shaking, with 0.01 M sodium thiosulphate until the yellow color almost disappears. Add 0.5 ml of starch solution and continue the titration, shaking vigorously until the blue color just disappears. Carry out a blank titration omitting the substance under examination (b ml). The volume of 0.01 M sodium thiosulphate in the blank determination must not exceed 0.1 ml.

Formula

$$\text{Peroxide value} = 10 \times \frac{(a-b)}{w}$$

Where,

w = weight of the substance in g.^[18]

VI] Specific Gravity

Estimation of specific gravity of oil is very important quality criterion for its assessment of purity. The specific gravity of a substance is a comparison of its density to that of water. The Specific Gravity (SG) - is a dimensionless unit defined as the ratio of density of the material to the density of water at a specified temperature. The actual weight of the empty specific gravity bottle is determined and followed by weight of water and the sample i.e oil. It is expressed as the ratio of density of the sample to the density of water at the specified temperature.

Procedure

Take accurate weight of an empty specific gravity bottle.



Fill the bottle completely with fish oil and weigh accurately.



Clean the bottle and fill completely with distilled water and weigh accurately.



Note down the temperature of the liquid.^[19]

Formula

$$\text{Specific gravity} = \frac{\text{weight of the oil}}{\text{weight of distilled water}}$$

B] Evaluation parameter of oil formulation

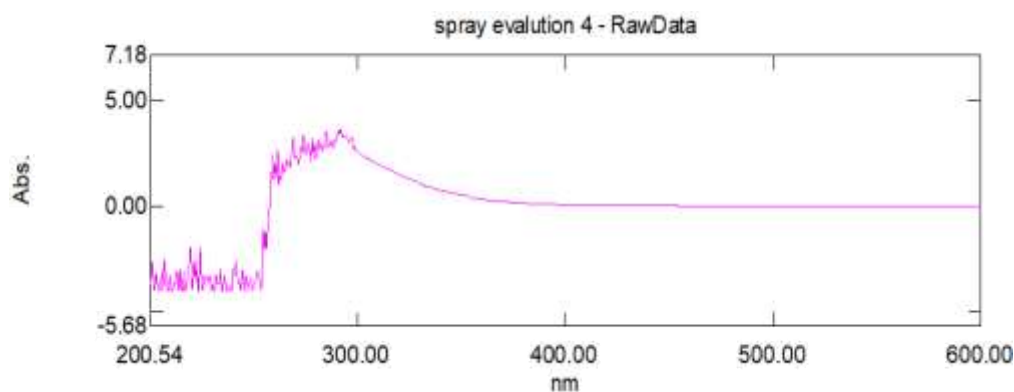
1] UV Analysis

Preparation of standard solution of oil formulation

Formulated oil 1 ml was accurately weighed and transferred in a 10 ml volumetric flask. ethanol was added up to the mark to obtain a concentration of 10µg/ml of Stock solution.

UV spectrum of formulated oil standard solution

Standard solution of formulated oil was used for a spectral scan of oil between 200-400nm using the solvent system as blank.^[20]



Graph 7: Uv spectrum of oil formulation.

2] PH measurement

The pH of the oil formulations was determined by using a digital pH meter by dipping the glass electrode completely into One ml of oil was dissolved in 100 ml of distilled water and it was placed for two hours into gel system so as to cover the electrode. Then instrument reading in terms of pH was noted. The measurement of pH of formulation was done and average values were calculated.

3] Viscosity

The viscosity measurement of formulations was performed to investigate their flow properties using Brookfield viscometer with spindle type S95, variable rotation speed (5,10,20,50 and 100 rpm) with sample volume of 20 ml at $37 \pm 0.50^\circ\text{C}$. At each speed, the corresponding readings were noted (cPs). The measurement of viscosity of formulation was recorded and average values were calculated.

4] Density

Dried and emptied Pycnometer was weighed. The sample were filled in it and air bubble were allowed to rise to top before inserting the stopper. Pycnometer was handled by the neck with one or two layers of paper between the fingers and the bottle to avoid expansion due to the heat of the hand. The proper value of the density was known by dividing the resultant weight of liquid by its volume in Pycnometer.^[21,22,23]

5] Drug content

One ml of oil solution was taken and its absorbance was determined using UV spectrophotometer after adequate dilution using water at 256 nm. Concentration was determined from the standard plot and the drug content was calculated as % of theoretical value.^[24]

Formula

$$\text{Drug Content} = \frac{\text{Actual Drug content}}{\text{Theoretical Drug content}} \times 100$$

RESULT AND DISUCSSION

Formulation and Evaluation of herbal burn and wound healing oil for diabetic patients was done.

Standardization of oils**Table 3: Standardization of oil.**

Sr. no	Physicochemical Parameter	Turmeric oil	Aloe vera oil	Tea tree oil	Lemongrass oil	Sandalwood oil
1.	Appearance	yellow	Pale yellow	Pale yellow	Bright yellow	Light yellow
2.	Odour	Characteristic	Pungent	Characteristic	Pungent	Characteristic
3.	pH	3.7	4.64	5.5	4.7	6.4
4.	Acid value	2.805	6.732	4.488	9.537	8.415
5.	Specific gravity	0.915	1.008	0.897	0.964	0.892

Physicochemical examination of prepared oil formulation**Table 4: Physicochemical examination of prepared oil formulation.**

Sr.no	Observation	Formulation
1.	Colour	Yellow
2.	pH	6.2
3.	Viscosity	43.2
4.	Homogeneity	Homogenous
5.	Consistency	Consistence

CONCLUSION

1. There is different antibiotic and antiseptic medication to treat burn.
2. The oil formulation containing turmeric oil, lemongrass oil, aloe vera oil, sandalwood oil, Tea tree oil.
3. Volatile and non-volatile oil used to develop oil formulation.
4. we have to prepare effective formulation that are used for no itching.
5. Burn and wound formulation are not available for diabetic patients so we have to prepare ayurvedic formulation.
6. Bioavailability of prepared herbal oil formulation is greater as compared to allopathic formulation.

ACKNOWLEDGEMENT

I would like to express my gratitude to my guide Ms. Inamdar M. B. for their valuable guidance and support in completing my project. I would like to extend my gratitude to Principal Dr. Santosh Ghule sir, Samarth college of pharmacy, Belhe.

REFERENCE

1. Rachmasari A, Kartini RA, Alviani G, Solihah I. Healing effect of Spray gel collagen extract from *Channa striata* bone on burn in rats. *Research Journal of Pharmaceutical Dosage Forms and Technology*, 2019; 11(4): 275-9.
2. Shakespeare P. Burn wound healing and skin substitutes. *Burns*, Aug. 1, 2001; 27(5): 517-22.
3. Shpichka A, Butnaru D, Bezrukov EA, Sukhanov RB, Atala A, Burdukovskii V, Zhang Y, Timashev P. Skin tissue regeneration for burn injury. *Stem cell research & therapy*, Dec., 2019; 10: 1-6.
4. Clark RAF. In: Clark RAF, Henson PM, editors. *Molecular and Cellular Biology of Wound Repair*. New York: Plenum., 1988; 3–33.
5. He JJ, McCarthy C, Camci-Unal G. Development of Hydrogel-Based Sprayable Wound Dressings for Second-and Third-Degree Burns. *Advanced NanoBiomed Research*, Jun., 2021; 1(6): 2100004.
6. Thakur R, Jain N, Pathak R, Sandhu SS. Practices in wound healing studies of plants. *Evidence-based complementary and alternative medicine*, Jan 1., 2011; 2011.
7. Mottahedin P, Haghighi Asl A, Khajenoori M. Extraction of curcumin and essential oil from *Curcuma longa* L. by subcritical water via response surface methodology. *Journal of Food Processing and Preservation*, Aug., 2017; 41(4): e13095.
8. Sulekha B, Avin G. Topical spray of silver sulfadiazine for wound healing. *J Chem Pharm Res.*, 2016; 8(7): 492-8.
9. Rachmasari A, Kartini RA, Alviani G, Solihah I. Healing effect of Spray gel collagen extract from *Channa striata* bone on burn in rats. *Research Journal of Pharmaceutical Dosage Forms and Technology*, 2019; 11(4): 275-9.
10. Kadam PV, Yadav KN, Bhingare CL, Patil MJ. Standardization and quantification of curcumin from *Curcuma longa* extract using UV visible spectroscopy and HPLC. *Journal of Pharmacognosy and Phytochemistry*, 2018; 7(5): 1913-8.

11. Singh A, Avupati VR. Development and validation of UV-spectrophotometric method for the estimation of curcumin in standardised polyherbal formulations. *Journal of Young Pharmacists*, 2017; 9(4): 491.
12. Patel PJ, Hinge M. Quantitative Estimation of Aloin in Aloe Vera and its Commercial Formulation by using UV Spectroscopy. *International Journal of Environmental Sciences & Natural Resources*, 2019; 21(3): 93-7.
13. Manjula BP, Joshi VG, Setty RS, Geetha M. Development and Validation of UV Spectrophotometric Method for Estimation of Tea Tree Oil in Bulk and Cosmeceutical Creams. *INDIAN JOURNAL OF PHARMACEUTICAL EDUCATION AND RESEARCH*, Jan. 1, 2021; 55(1): S285-92.
14. Manjula BP, Joshi VG, Setty RS, Geetha M. Simultaneous estimation of tea tree oil and neem seed oil in bulk and cosmeceutical formulation by UV spectrophotometry. *Int. J. Pharm. Sci. Res.*, 2021; 12: 3264-71.
15. Kumar GR, Chandrashekar BS, Rao MS, Ravindra M, Chandrashekar KT, Soundararajan V. Pharmaceutical importance, physico-chemical analysis and utilisation of Indian sandalwood (*Santalum album* Linn.) seed oil. *Journal of Pharmacognosy and Phytochemistry*, 2019; 8(1): 2587-92.
16. Liakos IL, Grumezescu AM, Holban AM, Florin I, D'Autilia F, Carzino R, Bianchini P, Athanassiou A. Polylactic acid—lemongrass essential oil nanocapsules with antimicrobial properties. *Pharmaceuticals*, Jul. 7, 2016; 9(3): 42.
17. Kumar GR, Chandrashekar BS, Rao MS, Ravindra M, Chandrashekar KT, Soundararajan V. Pharmaceutical importance, physico-chemical analysis and utilisation of Indian sandalwood (*Santalum album* Linn.) seed oil. *Journal of Pharmacognosy and Phytochemistry*, 2019; 8(1): 2587-92.
18. The Indian Pharmacopeia Commission, Ghaziabad, I: 142,151,144,151.
19. Geller DP, Adams TT, Goodrum JW, Pendergrass J. Storage stability of poultry fat and diesel fuel mixtures: specific gravity and viscosity. *Fuel*, Jan. 1, 2008; 87(1): 92-102.
20. Ma L, Lin Q, Lei D, Liu S, Song Y. Preparation and Properties of Sandalwood Essential Oil Microcapsules in Detergents. *Chemical Engineering Transactions*, Dec. 1, 2018; 71: 7-12.
21. Pawar IN, Rajput RR, Vaghani SS, Katara BV. Formulation and evaluation of topical spray containing anti acne agent. *Asian Journal of Pharmaceutical Technology and Innovation*, 2017; 5(24): 14-28.

22. Majid SA, Khorasgani MR, Emami SH, Talebi A, Gholami AM. Study of Diabetic cutaneous wound healing in rats treated with *Lactobacillus Casei* and its Exopolysaccharide. *International Journal of Advanced Biotechnology and Research*, Apr. 1, 2016; 7: 2083-92.
23. Sritharadol R, Nakpheng T, Wan Sia Heng P, Srichana T. Development of a topical mupirocin spray for antibacterial and wound-healing applications. *Drug development and industrial pharmacy*, Oct 3., 2017; 43(10): 1715-28.
24. Sulekha B, Avin G. Topical spray of silver sulfadiazine for wound healing. *J Chem Pharm Res.*, 2016; 8(7): 492-8.