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ORA- Experimental Research

Physicochemical and Phytochemical Evaluation of Aqueous and Alcoholic Extracts of *Tamraparna* (*Nicotiana tabacum* L.) and Development of Topical Gel Formulation

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ABSTRACT:

Background: *Tamraparna* (*Nicotiana tabacum* L.) leaves bioactive phytoconstituents exhibit a wide range of pharmacological activities. Method of extraction has high impact on the qualitative and quantitative analysis of extracts. Topical drug delivery systems primarily deliver the drug locally and provide increased patient compliance by reducing systemic side effects. Gels are convenient topical dosage forms having benefits of high aqueous component, permitting greater dissolution of drug and ease of application. **Objective:** To assess physicochemical and phytochemical properties of aqueous and alcoholic extracts of *Tamraparna* (Bhavyashree variety) leaves and to develop standardized gel formulation using alcoholic extract. **Materials and Methods:** Soxhlet extraction of *Tamraparna* dried leaves was done using water and ethanol as solvents and extracts were subjected to organoleptic, physicochemical, phytochemical (qualitative screening and quantitative estimation of total alkaloid) characterization using standard procedures. A topical formulation of gel was prepared using Carbopol 934 by one factor at a time (OFAT) approach employing extracts at various concentrations (1-6% w/w), which was then subjected to evaluation of physical parameters like extrusion, homogeneity, pH and preliminary skin sensitivity parameters. **Results:** Phytochemical screening of extracts divulged the presence of carbohydrates, reducing sugars, flavonoids, tannins, whereas alkaloids, proteins, steroids and cardiac glycosides were detected in alcoholic extract only. The total alkaloid content of extract was estimated 132.43 mg AE/100 g. Alcoholic extract exhibited excellent antioxidant activity that is 95.36% at a concentration of 250 µg/mL. Among various prepared formulations, 5% w/w gel exhibited proper physicochemical characteristics like good spreadability, extrudability, homogeneity, and neutral pH, and no skin irritation was observed during skin sensitivity studies**. Conclusion:** Alcoholic Extract of *Tamraparna* contains bioactive constituents exhibiting a more potent antioxidant potential than aqueous extract. 5% w/w gel formulation developed possesses good physicochemical characteristics indicating the suitability of the gel formulation as topical therapeutic preparation.

KEYWORDS: Alcoholic extract, Aqueous extract, Bhavyashree, Nicotine, Parikartika**,** Tamraparna.

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1. INTRODUCTION

Parikartika (Fissure in ano) is a commonly found ano-rectal condition. It is mainly characterized by a severe type of pain, burning sensation, along with discomfort during defecation that adversely affects the quality of life of the individual. It is mainly developed due to improper dietary and lifestyle habits and also as a complication of some therapeutics. [1] Though different treatment modalities are available for management of fissure-in-ano, recurrence and delayed healing are still a clinical challenge. Starting from Pre Modern Era 6th century BC till the modern era there are various procedures mentioned like warm sitz bath, ointments, polyherbal suppository (AYUHEAL suppository), *Yastimadhu* hydrogel, *Katuki lepa*, different sphincterotomy, fistulectomy, anoplasty etc. for fissure in ano treatment. [2-5] From topical agents like gels, ointments, creams, using gels has an advantage as they are non-sticky, show good spreadability, and do not show any stability issues. Thomas Graham in 1800s, coined the term “gel” emanated from “Gelatin”. In 1894, the term hydrogel was mentioned that have water attracting three dimensional network capacity useful in wound dressings and tissue engineering. [6] As per the United States Pharmacopoeia, Gels as a semisolid system consisting of a dispersion made up of either small inorganic particles or large organic molecules enclosing and interpenetrated by liquid. Topical delivery systems are important in the treatment of anorectal disorders because of their ability to provide localized action, decrease systemic side effects and improve patient compliance. Gels offer a suitable mode of delivery as they are non-greasy, easy to apply, have better spreadability and give good penetration of drug through the skin. For Topical drug delivery systems, skin is easily accessible route to deliver drug that passes through the stratum corneum (epidermis) and bypass first-pass metabolism. [7,9] In recent few years herbal formulations have become very popular due to their sophisticated utility and fewer side effects. But there are very few studies on development and standardization of herbal gel formulations with validated physicochemical and phytochemical characteristics.

Tamraparna (*Nicotiana tabacum* L.) is described in classical Ayurvedic texts with properties like *shothahara* (anti-inflammatory) as well as *vedanasthapana* (analgesic). [8-12] *Tamraparna* (*Nicotiana tabacum* L.) alcoholic extracted gel prepared is homogenous semisolid three-dimensional crossed-linked colloidal material which is syneresis-free, formed by synthetic gelling agent and follows a topical internal drug delivery system and colloidal single phase system in which organic molecules bound together by Vander walls forces. [7, 13] Phytochemical studies reported presence of alkaloids, flavonoids as well as tannins that may be responsible for its pharmacological activities. Nicotine, as the main alkaloid is associated with systemic toxicity but its controlled topical applications may offer local therapeutic actions with negligible systemic absorption. Some studies showed that *Tamraparna* (*Nicotiana tabacum* L.) have anti-tumor, anti-inflammatory, analgesic effects ,anti-oxidant, anti-ulcer, anti-cancerous, anti-fungal, haemostatic, anti-microbial properties, wound healing effect, anaesthetic effect as well as it has capacity to reduce the oxidative damage of organs and tissues. [8, 12-13] Even though *Nicotiana tabacum* contains nicotine which is biologically active alkaloid with known systemic toxicity, previously its controlled topical application has been explored for potential analgesic, anti-inflammatory as well as antimicrobial effects. This study focuses on external application, thereby minimizing systemic exposure at the same time utilizing its pharmacologically active constituents.

Therefore the current study aims to evaluate physicochemical and phytochemical properties of aqueous as well as alcoholic extracts of *Tamraparna* (Bhavyashree) leaves and to develop standardised topical gel formulation using its alcoholic extract.

The novelty of this study is precisely pointing out use of the Bhavyashree variety of *Tamraparna* (*Nicotiana tabacum* L.) because of its relatively higher concentration of alkaloids as a

determining factor influencing the phytochemical composition as well as formulation feasibility along with its development as gel formulation.

Aim: To standardize the dried leaves and extracts of Tamraparna (*Nicotiana tabacum* L.) and to formulate and evaluate a topical gel containing the selected extract.

Objectives:

a) Identification and authentication of raw drugs used for the preparation of *Tamraparna* (*Nicotiana tabacum* L.) alcoholic extracted gel.

b) Physicochemical and phytochemical analysis of *Tamraparna* dried leaves and extracts (aqueous and alcoholic).

Research question

What are the pharmacognostical, physicochemical, and phytochemical characteristics of the dried leaves and aqueous and alcoholic extracts of *Nicotiana tabacum* L., and can a standardized topical gel containing the selected extract be formulated and evaluated?

No formal hypothesis was framed because the study is exploratory and descriptive, involving pharmacognostical standardization, phytochemical characterization, and formulation development

2. MATERIALS AND METHODS

Ethical Considerations: Current study deals with physicochemical, phytochemical and pharmaceutical preparation of topical formulation and a preliminary skin sensitivity test in a healthy volunteer with informed consent has been performed. Current study does not employ any invasive procedures or adverse reaction to the study participant. So the study has been pursued under standard ethical consideration in conducting non-clinical research.

Materials: Soxhlet apparatus, glass rod, measuring flask, beakers, weighing machine, water bath, petri dish, crucible, spatula, filter paper, heating mantle, pair of gloves, mask, stirrer, sealing machine, UV chamber, packing material.

Methods:

Collection Condition

Tamraparna (*Nicotiana tabacum* L.) leaves of Bhavyashree variety (Bidi tobacco) were collected in dry form and stored at room temperature in the month of July 2024 from Agriculture Research station, Nippani, Karnataka. As shown in figure 1.

Figure 1 : Dried leaves of *Tamraparna* (Bhavyashree)

Identification and Authentication of Plant Material: The identification and authentication of the raw drugs were carried out on 29 July 2024 at the Ayurveda, Siddha, Unani Drug Testing Laboratory, a part of the Central Research Facility (CRF) at Shri B.M.K Ayurveda Mahavidyalaya, Belagavi, Karnataka with CRF code - CRF/Auth/434/2024-25 and Voucher specimen number- BMK/CRF/330/2024-25. Organoleptic characters and physicochemical analysis of raw drug [14, 15]

Colour, taste, odor, foreign matter, ash value, acid insoluble ash, water soluble extractive and alcohol soluble extractive were assessed as per The Drug and Cosmetic act 1940, form 50, rule no.160-D (f).

Extraction of alcoholic extract [16]: *Tamraparna* (*Nicotiana tabacum* L.) alcoholic extract was obtained using continuous hot extraction technique that is soxhlet extraction with Drug-solvent ratio 1:20 (w/v) at 60 degree temperature for 8 hours in which the solvent is repeatedly vaporized, condensed and passed through the sample to extract active constituents with 2.5 gm (5%) yield. Dried coarse powder of *Tamraparna* (*Nicotiana tabacum* L.) was put in porous thimble and ethanol was poured into round bottom flask and thimble. Then soxhlet extractor, condenser and thimble were assigned. After repeated cycles, when the solvent in the siphon tube becomes colourless, wait till the cooling of the apparatus. As shown in figure 2.Then

filter the solvent remained in round bottom flask using filter paper. A water bath was used to evaporate the filtered solvent. Then the concentrated alcoholic extract was collected. As shown in Figure 3.

Figure 2: Cooling of the apparatus

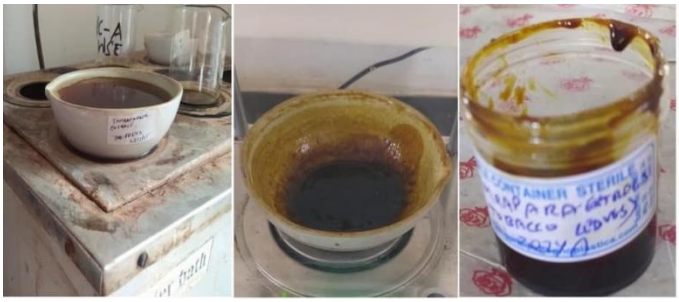

Figure 3 - Filtered solvent on water bath and Concentrated alcoholic Extract

Methods of evaluation of the *Tamraparna* alcoholic extract- The extract was analyzed by using standard qualitative and quantitative parameters. Organoleptic characters and physicochemical parameters etc at Central Research Facility (CRF) at Shri B.M.K Ayurveda Mahavidyalaya, Belagavi, Karnataka with reference no.-CRF/RM/288/2024-25.

Qualitative analysis [17-18]: Qualitative analysis was done for alkaloids by Dragendroff's test, tannins and flavonoids using lead acetate as reagent, carbohydrates by Molisch's test , reducing sugars by Benedict's test, monosaccharides by Barfoed's test, pentose sugar by Bial's Orcinol test, non-reducing sugar by Iodine test, hexose sugar by Selwinoff's test, proteins by Million's test, amino acids by Ninhydrin test, steroids by salkoviski reaction, tests for glycosides by Keller-Killiani test.as per The Drug and Cosmetic act 1940 ,form 50 ,rule no.160-D(f).

Quantitative analysis - total alkaloid content [19]: To know the amount of active principle present, total alkaloid content was measured.

Antioxidant activity [20]: DPPH (2.2-Diphenyl-1-picryl hydrazyl) radical scavenging assay was done at different concentrations to measure the antioxidant ability of the alcoholic extract.

Method of preparation of *Tamraparna* alcoholic extract gel: *Tamraparna* (*Nicotiana tabacum* L.) alcoholic extracted gel was prepared at KLE Society's KLE AYURVED PHARMACY (GMP certified unit), Khasbag , Belagavi, Karnataka.

Preparation of gel [21]: The leaves of *Nicotiana tabacum* L. (Bhavyashree variety) were dried in a herbal medicine dryer to decrease its moisture content. The dried leaves were transformed in coarse powder.

Pilot study

Base preparation: 1.067 gm of Carbopol 934, 100 ml of Distilled water, 0.005 gm of EDTA Sodium, 5 gm of Propylene glycol and 4 drops to 10 drops of Triethanolamine was mixed homogenously for 100 gm of base preparation.

Gel preparation for pilot study: For pilot study 0.1 gm, 0.3 gm, 0.4 gm, 0.5 gm, 0.6 gm of extracts were mixed with 10 gm of gel base separately to make 1% w/w, 3% w/w,4% w/w, 5% w/w, 6% w/w gel respectively on 17-02-2025 as shown in table 1 . 4 drops to 10 drops Triethanolamine were added with each homogenous prepared gel till pH-7 achieved. Physical analysis, extrudability, homogeneity, skin sensitivity test, etc., of all the prepared gel was done. As shown in Figure 4.

Table 1: Gel preparation for pilot study

Concentration	Gel base	gm of *Tamraparna* extract
1%	10 gm	0.1 gm
3%	10 gm	0.3 gm
4%	10 gm	0.4 gm
5%	10 gm	0.5 gm
6%	10 gm	0.6 gm

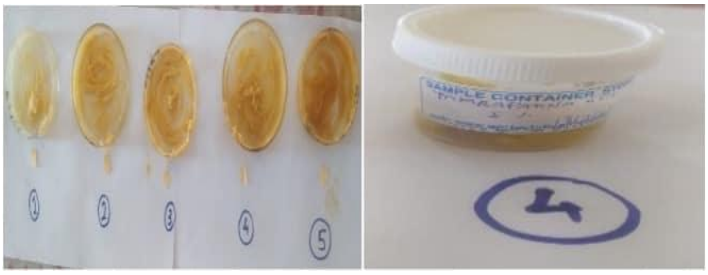

Figure 4 - Different concentration (1% w/w, 3% w/w,4% w/w, 5% w/w, 6% w/w) of gel preparation

Skin sensitivity test: As this study belong to gel formulation, physicochemical and phytochemical analysis, skin sensitivity test was conducted on healthy adult volunteer to know any adverse effect as per previous study. [22] Skin sensitivity test was done, following informed consent to assess local tolerability on 18-02-25 at 4:55 pm on left forearm anterior aspect in female. All 5 samples, 1 gm each were applied and waited till 20 minutes (5:15pm). No reaction/itching/no drowsiness or any other complaints were seen. After gel application cold sensation were felt at the applied points without any adverse reaction. Gel was removed after 20 minutes. No adverse reactions were observed during the observation period of next day. [22]As shown in Figure 5.

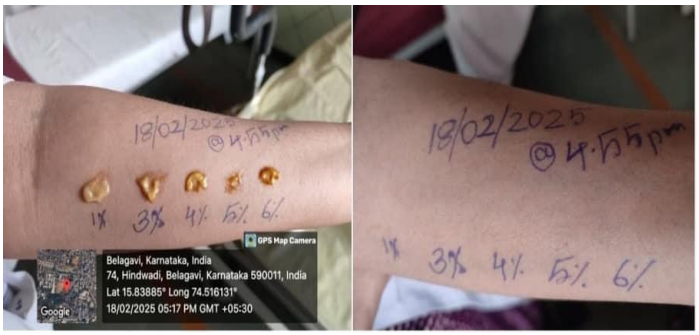

Figure 5 - Skin sensitivity test on the forearm done of different concentrations of gel

No skin irritation or other adverse effects were seen after the topical sensitivity test . According to physical analysis like spreadability, extrudability, homogeneity, skin sensitivity test etc,5% w/w gel was chosen for the study as shown in table 2.

Steps for *Tamraparna* (Bhavyashree) leaves alcoholic extract gel preparation at 5% concentration by Direct Dispersion Method

5% w/w concentration of *Nicotiana tabacum* L. extract was fixed because of physical analysis like spreadability, extrudability, homogeneity and satisfactory physicochemical, phytochemical characteristics in order to develop a pharmaceutically acceptable topical gel. As this study aims at herbal gel formulation, physicochemical and phytochemical analysis only. This study does not belong to dose-optimization study. Therefore, gel prepared by 5% w/w concentration can be considered as a preliminary formulation concentration.

Amount of Ingredients required to prepare gel are mentioned in table 2

Table 2: *Tamraparna* (Bhavyashree) leaves alcoholic extract gel preparation at 5% concentration

s.no.	Ingredients	Amount to prepare 100 gm of gel
1.	Carbopol 934	1.067 gm
2.	*Tamraparna* alcoholic extract	5 gm
3.	Distilled water	100 ml
4.	EDTA sodium	0.005 gm
5.	Propylene glycol	5 gm
6.	Triethanolamine	Few drops

1. Polymer dispersion - Carbopol 934, a synthetic polymer, weighs 1.067 gm sprinkled slowly into 60 ml of distilled water with continuous stirring and lump formation was avoided.
2. Hydration - Dispersion was left for a few hours to complete swelling up and uniform base preparation.
3. Drug solution – *Tamraparna* (*Nicotiana tabacum* L.) alcoholic extract, weighing 5 gm was dissolved in 5 gm of Propylene glycol and 30 ml of distilled water
4. Drug incorporation – the above drug solution was then added to the hydrated gel base and mixed.

5. Additives addition - EDTA sodium weighs 0.005 gm was mixed with 10 ml of distilled water and then added to the drug solution.
6. PH adjustment - Triethanolamine was added to the prepared solution to dropwise till the achievement of pH-7. This step is very important in gel formation whose base is Carpobol.
7. Final mixing of solution done to obtain smooth and homogeneous gel.
8. Deaeration was done.
9. Filling and packing of gel was done after sterilizing the tubes in UV chamber.

3. RESULTS

Qualitative and quantitative analysis were done of aqueous and alcoholic extracts of *Tamraparna* (Bhavyashree). *Tamraparna* (*Nicotiana tabacum* L.) alcoholic extracted gel was prepared at KLE Society's KLE AYURVED PHARMACY (GMP certified unit), Khasbag, Belagavi by using standard operating procedures. The analytical and pharmaceutical analysis are mentioned further below.

Identification of a drug

Identification of the drug outlined in table 3.

Table 3: *Tamraparna* (*Nicotiana tabacum* L.) leaves contents

Sanskrit name	Latin name	Family	Part	*Rasa*	*Guna*	*Virya*	*Vipaka*	*Karma*
Tamraparna	*Nicotiana tabacum* L.	Solanaceae	Leaves	*Tikta,Katu*	*Laghu, vyavayi, vikashi, tikshna*	*Usna*	*Katu*	*Kapha vata samak*

Foreign matter : In API a limit is mentioned for Foreign matter that is "Not more than 2%".

The given sample results 1.240% Foreign matter, which indicates that sample passes the specific limit as mentioned in Indian Pharmacopoeia and can be taken for further evaluation. As mentioned in table 4.

Table 4 : **Test reports of Organoleptic characters and physicochemical analysis of raw drug**

Macroscopic test	Result
Form	Leaves
Colour	Golden brown
Taste	Burning acrid
Odour	Penetrating disagreeable
Physiochemical tests	Result
Foreign matter	1.240%
Ash value	20.187%
Acid insoluble ash	5.760%
Water soluble extractive	39.693%
Alcohol soluble extractive	25.834%

Ash value and Acid-insoluble Ash: As per Indian Pharmacopoeia / British Pharmacopoeia, in leaves, the range of ash varies from 5-20% and acid insoluble ash below 5%. But different types of plant leaves have its specific limit. Ash value reveals total inorganic matter (natural and external minerals) and checks purity while acid soluble ash reveals silica or sand content (external contamination). Ash value of the given sample results 20.187% and acid insoluble ash results 5.760%. As mentioned in table 4.

Water and alcohol soluble extractive: This Quality control parameters for crude drug (leaves) indicates percentage of active constituents extracted by using water and alcohol as solvent. The percentage of water soluble extractive and alcohol soluble extractive in the *Tamraparna* (*Nicotiana tabacum* L.) leaves are 39.693% and 25.834% respectively. As mentioned in table 4.

Organoleptic characters: For identification and evaluation of dried leaves of *Tamraparna* ,sense organs were used which

results ,Golden brown in colour , Burning acrid in taste and Penetrating disagreeable Odour of the crude drug as mentioned in table 4

Qualitative analysis: *Nicotiana tabacum* L. leaves aqueous and alcoholic extracts were underwent Qualitative phytochemical analysis using standard pharmacognostic procedures. Carbohydrates were detected by Molisch's test, reducing sugars by Benedict's test, monosaccharides by Barfoed's test, pentose sugars by Bial's orcinol test, non-reducing sugars by acid hydrolysis followed by Benedict's test, hexose sugars by Seliwanoff's test, proteins by Biuret test, amino acids by Ninhydrin test, steroids by the Liebermann–Burchard test, flavonoids by the Shinoda test, alkaloids by Dragendorff's and Mayer's tests, tannins by Ferric chloride test, cardiac glycosides by the Keller–Killiani test, anthraquinone glycosides by Borntrager's test, and saponin glycosides by Froth (Foam) test. In respective tests , changes in colour of precipitate determines the presence or absence of phytochemical constituents. Standard pharmacognostic methods were used to conduct qualitative tests which does not require analytical instrument to intervene, As mentioned in table 5.

Quantitative analysis - total alkaloid content

Atropine was used as standard alkaloid to estimate the total alkaloid content present in 100gm of *Tamraparna* leaves as shown in table 6.

Table 5: Preliminary phytochemical screening in water and alcohol extracts and tests for glycosides

Phytochemical Constituent	Method/Test Used	Water Extract	Alcoholic Extract
Carbohydrates	Molisch's Test	Positive	Positive
Reducing Sugars	Benedict's Test	Positive	Positive
Monosaccharides	Barfoed's Test	Positive	Positive
Pentose Sugars	Bial's Orcinol Test	Negative	Negative
Non-Reducing Sugars	Hydrolysis followed by Benedict's Test	Negative	Negative
Hexose Sugars	Seliwanoff's Test	Negative	Negative
Proteins	Biuret Test	Negative	Positive
Amino Acids	Ninhydrin Test	Negative	Negative
Steroids	Liebermann–Burchard Test	Negative	Positive
Flavonoids	Shinoda Test	Positive	Positive
Alkaloids	Dragendorff's Test / Mayer's Test	Negative	Positive
Tannins	Ferric Chloride Test	Positive	Positive
Cardiac Glycosides	Keller–Killiani Test	Negative	Positive
Anthraquinone Glycosides	Borntrager's Test	Negative	Negative
Saponin Glycosides	Foam Test / Froth Test	Negative	Negative

Table 6 : Total alkaloid content

Sample code	Sample name	Type of extract	Results
RM/288	*Tamraparna* leaves (Tobacco)	Soxhlet Methanolic extract	132.43mg AE/100gm of sample

Antioxidant activity

The *Tamraparna* leaves extract showed significant DPPH radical scavenging activity. As with increase in concentration from 31.25 to 250 µg/mL, percentage inhibition increased from 72.89 % to 95.36%. The observed data shows high free radical

scavenging activity, indicates strong antioxidant potential. As shown in table 7.

Table 7: Antioxidant activity of alcoholic extract

Concentration µg/mL	% Radical scavenging activity
31.25	72.89
62.50	77.74
125.00	87.90
250.00	95.36
500.00	94.86
1000.00	94.10

Evaluation of gel

Appearance, spreadibility, extrudability and pH was evaluated.

a) **Spreadability:** On a glass plate 1.0 gm of the gel was placed in a 10 mm of diameter circle. Another glass plate was placed on previous one and sample was pressed with 500gm of weight. After 30 seconds, difference between diameters was calculated on a millimetre scale placed under the lower glass plate. [22,23]

b) **Extrudability:** On applying pressure on tube whatever amount of gel extruded that measures as Extrudability. [22,23]

c) **pH –** pH was determined with pH paper results 7.

4. DISCUSSION

The present study revealed that alcoholic extract of *Tamraparna* (*Nicotiana tabacum* L.) possesses a wide range of phytoconstituents likewise alkaloids, proteins, steroids, cardiac glycosides when compared to that of aqueous extract of the plant. This correlates with the earlier studies which states that alcohol is a semi-polar solvent in which both polar as well as non-polar bioactive compounds gets extracted very efficiently when compared to water alone. Organoleptic, physicochemical analysis, qualitative and quantitative analysis are the necessities to evaluate a drug preparation before its therapeutic use. According to Ayurvedic principles, Topical ayurvedic gels are enriched with botanical components from which absorption of beneficial plant substances into the body of skin occur that enables the direct delivery to the targeted area. [23]

Dried leaves of *Tamraparna* (Bhavyashree) organoleptic character reveals golden brown colour, burning acrid in taste and have a penetrating disagreeable odour. Its alcoholic extract shows golden brown colour and was semisolid. Its phytochemical analysis shows Foreign matter-1.240% , Ash value - 20.187%, Water soluble extractive -39.693% , Alcohol soluble extractive- 25.834%, which indicates good purity and rich presence of phytoconstituents like alkaloids, tannins, flavonoids etc. that may support suitability in therapeutic activity as well as good extraction in solvents outlined in table 4. Water and Alcohol extracts of *Tamraparna* (*Nicotiana tabacum* L.) leaves contain various phytochemicals from which alkaloids, proteins, steroids, cardiac glycosides are only present in alcoholic extract outlined in table 5. *Tamraparna* (*Nicotiana tabacum* L.) poses *tikta* (bitter), *katu* (pungent) *rasa*, *ushna virya* (hot potency), *laghu* (light), *tikshna* (sharp), *vyavayi* (penetrating/diffusing), *vikashi* (separating) *gunas* , balances *vata* and *kapha dosha*, *madak* in *prabhava*. [10-12,24] The alkaloids present in *Tamraparna* may generate a warming effect that can decrease blockage due to cold. The primary alkaloid Nicotine act on Nicotinic anticholinergic receptors, decreases the release of TNF-α, IL-1β, and IL-6 leads to temporary desensitization may act as anesthetic and analgesic drug. Nicotine which is main alkaloid of *Nicotiana tabacum*, interacts with nicotinic cholinergic receptors. Despite of its therapeutic potential, risk of systemic toxicity warrants careful usage. The current study utilized topical route in order to restrict systemic exposure and associated risks. Carbohydrates found in tobacco leaf extract possess antioxidant properties as COOH,-C- and C=O groups reduce free radicals by donating electrons. [24-26]

Quantitative analysis of *Tamraparna* Methanolic extract results total alkaloid 132.43mg AE/100 g of sample, showing the activity as comparable to atropine.outlined in table 6 [27]

Antioxidant activity of *Tamraparna* extract was measured by

DPPH and results in highest activity at 250 µg/mL of concentration that is 95.26% as shown in table 7, that indicates drug may reduce oxidative stress by neutralizing free radicals, prevents damage to the cell . [20]

Tamraparna (*Nicotiana tabacum* L.) alcoholic gel prepared has shown good spreadability, extrudability, neutral pH and may act as a good therapeutic drug due to the presence of bioactive constituents.

Selection of Bhavyashree variety was basically due to its comparatively higher alkaloid content which further augments the phytochemical richness as well as functional efficacy of the formulation. Apart from this topical gel enables localized delivery of drug, enhancing therapeutic outcomes while mitigating systemic exposure and related risks.

Strength: Current work reflects holistic and integrative approach of study by comparative evaluation of aqueous and alcoholic extracts phytochemically besides formulating them. Noteworthy strength is that Bhavyashree variety of *Tamraparna* (*Nicotiana tabacum* L..) is chosen for present formulation as it is found to contain increased levels of alkaloids thus making formulation phytochemically richer and therapeutically more promising. Quantitative estimation of total alkaloids in conjunction with antioxidant estimation authenticates pharmacological basis. Further systematic optimization of the gel concentration using OFAT approach and evaluation of physicochemical parameters of the developed formulation aids in reproducibility as well as standardization of the developed formulation.

Limitations: Existing research study was mostly concentrated on physicochemical plus phytochemical assessment of aqueous plus alcoholic essences of *Tamraparna* (*Nicotiana tabacum* L.) along with the growth of a topical gel solution. In present study phytochemical screening was performed only on *Tamraparna* alcoholic extract and not on the final gel formulation. So retention of individual bioactive constituents in finished gel was not directly confirmed. Further phytochemical characterization of formulated gel is warranted in future studies. Advanced logical strategies likewise HPLC, HPTLC, LC-MS, or chromatographic fingerprinting were not done for recognition as well as quantification of particular pen substances. Thorough safety and security assessment criteria consisting of hefty steel evaluation, microbial lots, chemical deposit aflatoxins, together with chemical efficiency screening, were not executed. Apart from this, comprehensive solution analysis research studies likewise rheological analysis, medicine web content uniformity, sped up security screening, in-vitro medicine launch as well as skin permeation research studies were past the range of today job. Analytical contrast in between aqueous and also alcoholic essences was not carried out. As a result better research studies entailing sophisticated logical personality, security analysis, security examination as well as preclinical as well as medical examinations are needed to develop the high quality, security as well as restorative capacity of the created formula.

5. CONCLUSION

The present study demonstrates that the alcoholic *Tamraparna* (*Nicotiana tabacum* L..) (Bhavyashree variety) extract is richer in phytochemical compounds and higher in antioxidant activity than aqueous extract. 5% w/w topical *tamraparna* dried leaf gel being developed over satisfactory physicochemical properties elicited favourable topical profile allowing for a localized drug delivery. 5% w/w topical gel obtained overlaps a perspective formulation of topical therapeutic further future preclinical and clinical studies are needed to prove safety and efficacy.

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