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Open Access **Research Article**

Phytochemical Screening of alcoholic extract of Thuja Occidentalis Leaves for Formulation and Evaluation of Wound Healing Ointment

Keshav Bharti*, Manmohan Sharma, Girish Kumar Vyas, Shailesh Sharma

School of Pharmaceutical Studies, Faculty of Health Sciences, Dr. K. N. Modi University, Newai, Rajasthan

ABSTRACT

Herbal cosmetics are concoctions that are used to beautify and improve the look of humans. The goal of this study was to create and assess a herbal ointment including leaves extract made with alcohol for wound healing. Recent studies in several regions of the world have revealed that t. occidentalis and its active component thujone have a high potential for treating a variety of health problems. T. occidentalis preparations can effectively combat microbial/worm infection. It has antioxidant, anticancer, and anti-inflammatory properties. In addition to these effects, it has insecticidal, molluscicidal, and nematocidal efficacy against certain pests.During the research period, the formulated ointment demonstrated good consistency and spreadability, homogeneity, pH, non-greasy, and no signs of phase separation. Stability characteristics such as visual appearance, nature, viscosity, and aroma of the prepared ointment revealed no significant modification over the research period.

Keywords: Thuja occidentalis, Antimicrobial, Formulation, Evaluation, Phytochemicals screening etc.

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***Address for Correspondence:**

School of Pharmaceutical Studies, Faculty of Health Sciences, Dr. K. N. Modi University, Newai, Rajasthan

INTRODUCTION

Herbal therapy predominates in both conventional and alternative medicine performed in both developing and industrialised nations. The enormous interest in plant-derived pharmaceuticals stems from the notion that plants are safe, trustworthy, and have fewer adverse effects. A review of the literature suggests that traditional plant medications are useful for a variety of skin disorders and wound healing [1]. India is the world's greatest producer of herbal medicine and is appropriately known as the "Botanical Garden of the World." There are several acknowledged indigenous systems of medicine in India, including Ayurveda, Siddha, Unani, and Homeopathy; Naturopathy is used for human health care [2].During the research period, the formulated ointment demonstrated good consistency and spreadability, homogeneity, pH, non-greasy, and no signs of phase separation. Stability characteristics such as visual appearance, nature, viscosity, and aroma of the prepared ointment revealed no significant modification over the research period[3-4].

There are various reasons for the ongoing appeal of traditional pharmaceuticals, one of which is their ease of access in comparison to contemporary treatments, in addition to the negative effects of synthetic drugs [5]. Certain European and eastern nations have been studying the usage of plants for millennia and have been in practise. Great work has been done that is beyond the grasp and awareness of the average person. With the technologically advanced lifestyle of the twenty-first century, human pains are taking on new identities. The fundamental herbs contain the solution with no side effects and efficient therapies, and the best part is that herbal medicine may be used by anybody of any age [6].

Plants and herbs are widely utilised for medicinal reasons to cure a wide range of illnesses and disorders. Thuja occindentalis, popularly known as arbour vitae, is a member of the Cupressaceae family. This eurpeon tree is commonly utilised in both homoeopathy and phytotherapy [7].

Thuja is also known by other names such as northern white cedar, eastern white cedar, thuja, and so forth. Thuja occidentalis f. buchananii (Spath) Rehder is another variety.

Thuja occidentalis subsp. cristata (Carriere) Thuja occidentalis f. filiformis (Beissner) Rehder, Thuja occidentalis f. lutescens (Beissner) C. K. Schied, Thuja occidentalis f. pendula (Gordon) Beissner Active elements of the plant can be extracted using several extraction procedures, such as microwave aided hydrodistillation extraction, steam distillation, and hydro distillation [8-9].

The Thuja plant has a variety of components with varying biological activities. Borneol, camphene, fenchone, limonene, and thujone are examples of essential oils (can be isolated from the leaves and steam). Flavonoids such as catechine, myricetin, quercetin, and myrecitrin are active components, as are tannic acid, polysaccharides, and proteins. Plant extracts have anti-diabetic, anti-bacterial, nematicidal, anti-viral, and larvicidal action. There are commercially available formulations for the treatment of warts and acne. Thuja gel, thuja ointment, and oral formulations are also available for the treatment of a variety of acute and chronic diseases [10-11].

Taxonomical classification of Thuja occidentalis.

Table 1: Taxonomical classification of Thuja occidentalis [10]

Sr. No.	Classification
Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Coniferophyta
Class	Pinopsida
Order	Pinales
Family	Cupressaceae
Genus	Thuja
Species	Occidentalis

The objective of the present study was to formulate and evaluate formulation of alcoholic extract of thuja occidentalis leaves Ointment.

MATERIALS AND METHOD

Plant collection and authentication are the first steps in the preparation process. According to my study objectives, this procedure progresses through extraction and finally chemical analysis. The preparation is then developed into a formulation and evaluated.

Plant Collection and authentication:

Thuja occidentalis leaves were collected from a plantation in Newai, Tonk, Rajasthan. It was authenticated by Mr. Manmohan Sharma Associate professor and Dr. Shailesh Sharma, Professor Pharmacy department, Dr. K. N. Modi University and agriculture department of Dr. K. N. Modi University, Newai, Rajasthan.

Ash value determination:

The ash value of the chosen plant was determined using a muffle furnace on the campus of Dr. K. N. Modi University in Newai, Rajasthan, using the procedure outlined in the article reference no. [15].

Preparation of extract:

Using an electric mixer, dried fruits (50g) were mashed with the outer shells to make a coarse powder. This was then placed in a muslin bag and treated to Soxhlet extraction with ethanol (90 percent) as the solvent for 24 hours at 60° C. The rotary evaporator with a standard temperature of 45° C was used to remove the solvent from the Ethanolic Extract of Thuja occidentalis leaves [12].

Chemicals and Equipment's

Ferric chloride, glacial acetic acid, dilute ammonia solution, concentrated sulfuric acid, chloroform, Fehling's A and B solution, sodium picrate solution, biuret's reagent (purchased from R. S. Enterprises, Jaipur, Rajasthan).

Electric mixer, Soxhlet apparatus, Rotary evaporator, beakers, muslin cloth, weighing scale, test tubes, test tube holder, digital water bath are among the equipment used.

Phytochemical analysis:

Qualitative phytochemical assays were used to evaluate the presence of phytochemical elements in the test material.

A. Test for alkaloids

In 2ml of methanol, 20mg of ethanolic extract of thuja occidentalis leaves was dissolved. A few drops of 1% HCl were added to it. The mixture was then heated in a water bath and cooled before being treated with a few drops of Wagner's reagent. Turbidity and precipitation were measured in the sample [13].

B. Test for tannins

In a test tube, 20mg of ethanolic extract of Thuja occidentalis leaves was diluted in 1ml of distilled water, and 1-3 drops of Ferric chloride were added. The mixture was then examined for blue or green colour [13].

C. Test for cardiac glycosides

In 1ml of glacial acetic acid, 20mg of ethanolic extract of thuja occidentalis leaves was dissolved, and 1-2 drops of ferric chloride solution were added. Along the walls of the test tube, 0.5ml of concentrated sulphuric acid was gently added. A brown ring at the interface suggested the presence of a cardenolide deoxy sugar [13].

D. Test for saponins

40 mg of thuja occidentalis leaf ethanolic extract was diluted in 5ml of distilled water and forcefully shaken until a stable persistent froth was produced. The foam was combined with three drops of olive oil and rapidly agitated before being examined for emulsion [12-13].

E. Test for flavonoids

In 1ml of distilled water, 20mg of ethanolic extract of thuja occidentalis leaves was dissolved. It was treated with 0.5ml of weak ammonia solution. Later, conc. sulphuric acid was added. The presence of flavonoids was shown by the presence of a yellow colour. After letting the solution to rest for a few minutes, the yellow colour faded [12-13].

F. Test for terpenoids

In 1ml of chloroform, 20mg of ethanolic extract of thuja occidentalis leaves was dissolved, and 1ml of strong sulphuric acid was added. Terpenoids were detected by a reddish-brown discoloration at the interface [13].

G. Test for carbohydrates[12-13]

Fehling's test: A few drops of thuja occidentalis leaf ethanolic extract were heated with Fehling's A and B solutions. The presence of carbohydrates is indicated by the appearance of orange red precipitate.

Test for lactones

Baljit's test: Sodium picrate solution was applied to an ethanolic extract of thuja occidentalis leaves. The existence of a lactone ring is indicated by the presence of a yellow to orange colour.

H. Test for proteins

Biuret test:

To 2ml of extract, 2ml of Biuret reagent was added. It was thoroughly shook and warmed in a water bath. The presence of proteins is indicated by the appearance of red or violet colour.

I. Fixed oils and fatty acid

Spot test: The presence of fixed oil and fats was detected by a prepared spot on the filter paper with the test solution and oil staining on the filter paper [13].

Ointment preparation

After the phytochemical study competition, prepare for the ointment preparation. Some formulas were created specifically for the creation of wound healing ointment. The extracted material was dried in a rotatory drier and kept at 2-80 C temperature for subsequent formulation with the proposed recipe.

Table 2: Formula of ointment preparation

Sr. No.	Component	Function	Content % (w/w)
1	Extracted yield	For wound Healing	3
2	White petroleum	Oil base of o/w emulsion	25
3	Stearyl alcohol	Hydrophobic oil soluble component, Used as an emollient, emulsifier and thickener	25
4	Propylene Glycol	Hydrophilic viscous liquid used in the aqueous phase to increase viscosity	12
5	Solium lauryl sulphate	Surfactant/emulsifier	1
6	Water	Aqueous base of o/w emulsion	34

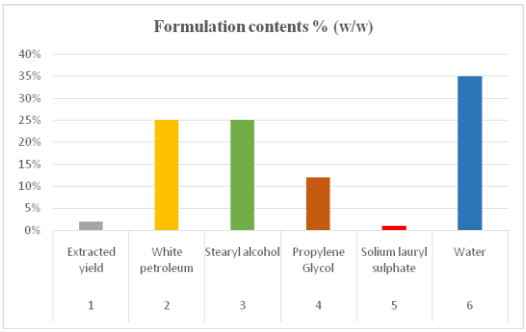

Figure 1: Formula Chart presentation for ointment preparation

Evaluation parameters: Evaluation parameters of the ointment are given below.

Colour and Odour

Visual inspection was used to analyse physical factors such as colour and odour [14].

Consistency

There is no greedy behaviour noticed [14].

PH

The PH of the produced formulations was determined using a digital PH metre. The ointment solution was made with 100ml of distilled water and placed aside for 2 hours. The solution's PH was tested in triplicate and the average value was computed [14].

Spreadability

The spreadability was measured by sandwiching an excess of sample between two slides that were squeezed to equal thickness by applying a fixed weight for a fixed period of time. Spreadability was determined by the time it took to separate the two slides. The shorter the time required to separate two slides, the better the spreadability [14].

Spreadability was calculated by following formula

$S=M\times L/T$

Here,

S= Spreadability

M= Weight tide to the upper slide

L= Length of glass slide

T= Time taken to separate the slides

Extrudability

The formulas were placed within a collapsible tube container. The weight of formulations necessary to extrude 0.5cm of ointment ribbon in 10 seconds was used to measure extrudability [14].

Diffusion studies

Agar nutritional medium was prepared for the diffusion investigation of formulations. A hole board was set in the centre of the medium, and formulae were inserted in it. The time it took formulations to diffuse through the system was recorded. (60 minutes later) [14].

LOD

LOD was established by putting the formulations on petri dishes on an oil bath and drying them at 105°C.

Solubility

Boiling water soluble; miscible with alcohol, ether, and chloroform.

Washability

After applying the formulations to the skin, the ease of washing with water was tested.

Non irritancy Test

The produced formulations were applied to human skin and the impact was noticed.

Stability study

The formulation's physical stability was tested for four weeks at three different temperatures: 2°C, 25°C, and 37°C. Within four weeks, the formulations were shown to be physically stable at varied temperatures, namely 2°C, 25°C, and 37°C [14].

RESULT AND DISCUSSIONS

As the objective of the present study was to formulate and evaluate formulations of Ointment from ethanolic extract of thuja occidentalis leaves and evaluate the efficacy with different quality determination tests.

Ash value determination

Ash value determination is known as the physicochemical parameter's evaluation of thuja occidentalis leaves.

Table 3: Ash value determination

S. No.	Physicochemical parameters	Observation
1	Loss of drying	8.00%
2	Total Ash value	3.08%
3	Water soluble ash	0.85%
4	Acid insoluble ash	1.5%
5	Foreign matter	Nil

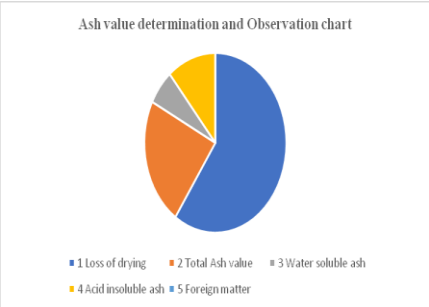

Figure 3: Ash value determination and Observation chart

Preparation of extract:

Dried leaves (50 gramme) were extracted in Soxhlet using ethanol (90 percent), and the yield obtained was 6% (w/w), 3 grammes.

Table 3: Extraction yield

S. No.	Crude drug quantity Taken	Yield Obtained
1.	50 Grams	(3grams) or 6%

Phytochemical screening:

Qualitative phytochemical assays were used to evaluate the presence of phytochemical elements in the test material.

The presence of carbohydrates, alkaloids, glycosides, phenolic compounds and tannins, saponins, flavonoids, fixed oils, and fat test was discovered in the plant extract of Thuja occidentalis leaves (Table 3.3).

Table 4: Phytochemical evaluation from ethanolic extract of thuja occidentalis leaves

Phytochemical tests			
S. No.	**Phytochemical constituents**	**Test**	**Results**
1	Carbohydrate	Fehling's test	**Positive**
2	Alkaloids	Mayer's test, Wagner's test, Hager's test	**Positive**
3	Glycoside	Molish' test,	**Positive**
		Keller-Killani test Positive with con. H2So4	**Negative**
4	Phenolic compound and Tannins	Lead acetate test, ferric chloride test, using ferrous sulphate and sodium potassium tartrate	**Positive**
5	Saponins	Foam test	**Positive**
6	Flavonoids	NaOH test, lead acetate test	**Positive**
		With H2So4, zinc test	**Negative**
7	fixed oils and fat test	Spot test	**Positive**

Evaluation of Ointment

The current investigation was carried out to prepare and assess formulations. The herbal extracts were produced using the Soxhlet method. The physicochemical characteristics were investigated, and the findings for spreadability, extrudability, washability, solubility, loss on drying, and other qualities were found to be good.

Table 5: The physicochemical properties of Ointment

S. No.	Characteristics	Formulation 1	Formulation 2
1	Colour	Pale green	Pale green
2	Odour	Characteristic	Characteristic
3	Consistency	Smooth	Smooth
4	P^H	6.1	6.4
5	Spreadability(seconds)	7	8
6	Extrudability	0.4 gm	0.3 gm
7	Diffusion study (after 60 min)	0.7 gm	0.6 gm
8	Loss on drying	0.2%	0.3%
9	Solubility	Soluble in boiling water, miscible with alcohol, ether, chloroform	Soluble in boiling water, miscible with alcohol, ether, chloroform
10	Washability	Good	Good
11	Non irritancy	Non irritant	Non irritant
12	Stability study	Stable	Stable

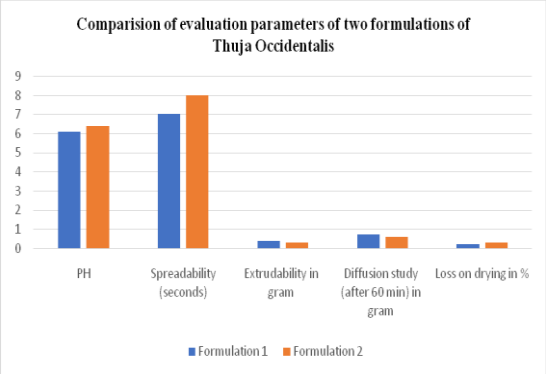

Figure 5: Comparison of physicochemical parameters of formulations of Thuja Occidentalis

In addition, the formulations were subjected to a four-week stability study at various temperatures such as 2°C, 25°C, and 37°C. There were no differences in spreading ability, diffusion study, or irritating impact.

So, some experimental results for the herbal plant thuja occidentalis and an ointment production and assessment are presented below. The overall findings were determined to be satisfactory. In the future, several animal-related research techniques will be carried out for this task. As a result, this is my future outlook on this job.

CONCLUSION

The current study concludes that Thuja occidentalis extraction is necessary in order to develop better, safer, and more cost-effective medications for treating various bacterial infections. This study demonstrates that Thuja occidentalis has a variety of phytochemicals as an active ingredient, which produce good results against antibacterial activity and have a high potential as an antibacterial agent. These claims have already been made by other researchers. When prepared as a topical ointment, it might explain the reported efficacy in the traditional usage of the plant in the treatment of common skin disorders. The anti-wounding properties of Thuja occidentalis herbal ointment. This will be researched more for my future studies. The finished product spread easily on the skin's surface, had no irritating impact, diffused effectively, and was stable at varied temperatures. It is also necessary to do study on phytochemical and pharmacological issues. However, with ongoing study, it would be easy to design new formulations.

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