

(RESEARCH ARTICLE)



Formulation and evaluation of herbal ointment using leaves of *Thuja orientalis* L. Leaf extract

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Magna Scientia Advanced Research and Reviews, 2021, 03(02), 001–008

Publication history: Received on 26 September 2021; revised on 28 October 2021; accepted on 30 October 2021

Article DOI: <https://doi.org/10.30574/msarr.2021.3.2.0074>

Abstract

The study of diseases and their treatment are important part of our ancient time worldwide. The knowledge of medicinal plants must have been accumulated in the course of many centuries. Herbal plants are huge sources of neutraceuticals, prevent the different disease or maintain healthy life. Plant produces primary metabolites for their basic survival and secondary metabolites for their ecological, taxonomical and biochemical differentiation and diversity. Herbal medicine prepare different part of plant are used. Herbal drug is design as the alternative formulation for the external use in the form of ointment. For the clinical use the herbal ointment apply externally on human body. The main aims of this research are preparation of herbal ointment from the *Thuja orientalis* L. Plants used as antibacterial activity. Under this research work, it is design as herbal ointment with the help of Ethanolic extract of *Thuja orientalis* L. and determines the anti bacterial capacity on it. In vitro study the antibacterial activity of the Ethanolic extracts of dried leaves of *Thuja orientalis* L. are determine by using the Agar cup plate method versus different bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* etc. By blending the Ethanolic extract of *Thuja orientalis* L. (10 % w/w) into aqueous cream we formulated herbal ointment. The emulsifying agent and simple ointment bases are added in above herbal ointment for increase the antibacterial effectiveness during in vitro evaluation. *Thuja orientalis* L. (Family Cupressaceae) are broadly used in traditional system of medicine throughout different part of India, China, Russia, Korea, Japan, India, Florida and Iran. It is used in the treatment of diarrhea, cough, cold, bronchitis, and upper part repertory infection. The current study deals with the determination of formulation and evaluation of herbal ointment using *Thuja orientalis* leave extract.

Keywords: *Thuja orientalis*; Herbal ointment; *Staphylococcus aureus*; *Escherichia coli*; Cupressaceae

1. Introduction

Herbal drugs are important resource, especially in developing countries, to treatment of different diseases. India is the largest producer of herbal medicine and it is rightly called the “Botanical Garden of the world”. India has number of approved indigenous systems of medicine viz-Ayurveda, Siddha, Unani, Homeopathy; Naturopathy is applied for the health care of mankind [1]. Ayurveda derived from two words, Au means life and Veda means knowledge.

One fourth of modern medicine is derived from plant base origin. Approximately 60% to 80% of the world’s populations still depend on traditional medicines for the treatment common diseases. Herbal plants are economical and more acceptable to most of the population in the world. Even in many of the modern medicines, the basic composition is derived from medicinal plants and has become acceptable for easy availability, least side effects, and low prices, environmental friendlily and lasting curative property compared to allopathic medicine [2-3]. The World Health Organization (WHO) has defined traditional medicine as “the sum total of all the knowledge and practices, whether explicable or not, also used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying

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exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing" [4]. In one of the studies of the World Health Organization, it is estimated that 80 per cent of the population of developing countries rely on traditional plant-based medicines for their health requirements [4-7]. There are several factors for the continued popularity of traditional drugs and one is their ready availability as compared to the modern medicines besides the adverse effects of synthetic drugs [8]. World Health Organization (WHO) has stressed the need to promote the indigenous systems of medicine among the rural population of the third World Countries [9]. The practice of complementary and alternative medicine is now increasing in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections [10-11]. In recent scenario herbal ointment is more popular formulation used for external application. The conveying of drugs through the skin is an encouraging concept because of ease of access, large surface area, vast exposure to the circulatory and lymphatic networks and protective nature of the treatment [12]. Instead of the alternative formulation like herbal medicine may also be prepared in the form of ointment. These ointments mention a viscous semisolid preparation applied externally on body surfaces area such as the skin, mucus membranes of the eye, vagina, anus, and nose etc. These ointments have specific medicinal values. The medicated ointments contain a medicinal ingredient mixed, suspended or emulsified in the ointment base. Herbal ointment applied externally such as antipruritic, keratolytics, protectants, antiseptics, emollients and astringents. Ointment bases are mainly free from water and generally contain one or more chemical in suspension or solution or dispersion form. Hence Ointment bases may be different types like absorption bases, dehydrating hydrocarbon water soluble type [13].

Herbal plants have ability for the formation of secondary metabolites such as steroids, phenolic substances, flavonoids, alkaloids, glycoside etc. These secondary metabolites are used for treatment of many diseases. The secondary metabolites provide a rich biogenic source for novel drug discovery. The metabolites produced by different plants vary from each other. *Thuja orientalis L.* belonging to the family Cupressaceae brought its importance for its different traditional uses throughout India. It is commonly known as Morepankhi or Thuja. On folklore study *Thuja orientalis L.* have various therapeutic importances. It is an ornamental plant. It is native to north western China and distributed in Russia, Korea, Japan, India, Florida and Iran. It is also cultivated in different parts of the world in parks, gardens, home yards etc. *Thuja orientalis L.* leaves contain essential oil its toxic material α -thujone has insecticidal, anthelmintic property to treat parasitic worms. And disrupt neurological signal in the brain, so ingestion of essential oil can cause death [14]. *Thuja orientalis L.* leaves have been traditionally used as antibacterial, antipyretic, antitussive, astringent, emollient, expectorant, febrifuge, haemostatic, stomachic and diuretic. The shoot is flat, leaves are scale like and the leaves are arranged in flattened fan shaped growing with resin glands [15]. It is a small to short sized tree growing to height 15-20 meter. It is aromatic and medicinal plant widely used in traditional medicine and aromatherapy. This plant is bright green in color but turns brownish or coppery orange in winter. It is used in treatment of acute and chronic infections of the upper respiratory tract, chronic tracheitis, gout, and diarrhea and hair loss. Leaf contains huge amount of essential oil used to treat tonic, diuretic, antipyretic, it is also used as anti-tussive, expectorant, anti-inflammatory, anti-bacterial, anti-fungal, anti-oxidant and also occasionally used for treating disease of skin, blood, gastrointestinal tract, kidney, brain, spongy tumors. Also used for hepatoprotective activity [16]. Fresh leaves contain 65% thujone, 8% isothujone, 8% fenchone, and 5% sabinene. Its twigs and leaves contain 0.12% essential oil containing pinene and probably caryophyllene. [17]. Other monoterpenes are namely carvotanacetone, organol, organene, myrcene and camphor.

Today in the modern era, pathogenic bacteria have developed resistance against existing antibiotics because of the extensive use of antimicrobial drugs against infectious diseases. So some of the active compounds prohibit growth of the disease-causing microbes either singly or in combinations. For a long period of time plants have been a precious source of natural products which are used to maintain human health, especially in last decades with more extensive studies for natural treatments. There is a continuous and immediate need to invent the new antimicrobials compounds with the varied chemical structure and innovative mechanisms of action for new and re-appearing infectious diseases. So scientists are increasingly turning their attention to community medicines, looking for new leads to develop better drugs against microbial infections. Considering that extracts of *Thuja orientalis L.* show broad spectrum antimicrobial activity. The aim of the study was to show that *Thuja orientalis L.* has antibacterial activity and also has high potential as antibacterial agent when synthesized as ointment for topical use. Ethanolic extract of *Thuja orientalis L.* *Thuja orientalis L.* belonging to the family Cupressaceae brought its importance for its different traditional uses throughout India. It is used in the treatment of various diseases such as colds, angina, pharyngitis, otitis media, sinusitis, bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinoma, amenorrhea, rheumatism and bronchitis. A yellow dye is obtained from young branches [18]. The aim of the current study deals the formulation and evaluation of herbal ointment using *Thuja orientalis L.* leaves extract.

2. Material and methods

Fresh leaves parts of *Thuja orientalis L.* were collected from fields of Chandeshwar, district of Azamgarh, Uttar Pradesh, India in the month of November 2017 and authenticated by DR. A.N Shukla, government of India, botanical survey of India Allahabad. Uttar Pradesh, India. A voucher specimen has been preserved in Department of Natural Product, Pharmacy College, Azamgarh, Uttar Pradesh, India for future reference (Voucher specimen no.thuja2017/11). The leaves parts were dried under shade and powdered (40 mesh size) and stored in airtight containers. The macroscopic characters were studied as per given procedure in WHO guidelines on quality control methods for medicinal plants materials [19].

2.1. Macroscopical studies

The leaves of the plant were studied for their macroscopic characters such as color, odour, taste, shape and size of the leaf.

2.2. Physicochemical studies

The loss of drying, ash value (total ash, acid insoluble ash, water soluble ash [20-21], swelling index[22-23], fluorescence analysis[24-25], extractive values (petroleum ether 60-80 °C, ethanol, chloroform, aqueous), foreign matter were determined according to the official methods of ayurvedic pharmacopoeia of India[26--29], were performed according to the official methods prescribed in Indian Herbal Pharmacopoeia[30]. and the WHO guidelines [31].

2.2.1. Test microorganisms

The microorganisms used for the study were *Staphylococcus aureus* and *Escherichia coli*. In this study, multi drug resistant wound separates bacteria from pathology, Civil Line, Azamgarh were used. The bacterial strains were raised and managed on Mueller Hinton agar at 37 °C.

2.2.2. Microbiological media

Chemicals and standard drugs Mueller Hinton Agar and Nutrient broth are collect from the Chemical store of the Pharmacy College, Azamgarh. Gentamicin ointment (1mg of Gentamicin in the form of Gentamicin Sulphate), obtain by medical store of MahaMrityunjai Hospital, Azamgarh.

2.2.3. Preparation of Ethanolic extract of *Thuja orientalis L.* (EETO). Leaves

The sun-dried leaves of *Thuja orientalis L.* powdered 4g of milled leaves powder are extracted with ethanol by maceration for 48 hr. The extracts are filter and concentrate using evaporator at 35°C to obtain semisolid extract. The extracts are stored in a desiccator. (EETO). Prepare at different concentrations of 500mg/ml, 250mg/ml and 100mg/ml.

2.2.4. Evaluation of antibacterial activity of Extract

The antibacterial activity of the Ethanolic extract of the leaves of *Thuja orientalis L.* (EETO).at concentrations of 100mg/ml, 250mg/ml and 500mg/ml were determined using the cup plate method. A molten Mueller Hinton agar stabilized at 45 °C was seeded with 0.1 ml of a 24 h broth culture of the test organism (*E. coli* and *S. aureus*) containing approximately 10⁸cfu / ml in a sterile Petri dish and allowed to set. Wells of 6mm diameter were created with a sterile cork borer and filled to about three-quarters full with solutions of the Ethanolic extract of the leaves of *Thuja orientalis L.* (EETO).The plates were pre-incubated for 1 h at room temperature to allow for diffusion of the solution and then incubated for 24 h. The zones of inhibition were measured (mean, n=2). Streptomycin and Gentamycin were used as positive and negative controls respectively. The in vitro bacterial response to the extract are evaluate using the diameter of the zones of inhibition as follows; resistant: 10mm and below, intermediate: 11-15mm and susceptible: 16mm and above.[32]

2.2.5. Preparation of Ointments

Three topical ointment bases of varying degrees of aqueous/anhydrous character namely simple ointment BP, emulsifying ointment BP and aqueous cream BP were prepare by fusion method. In this method the constituents of the base were placed together in a melting pan and allowed to melt together at 70°C. After melting, the ingredients were stirred gently maintaining temperature of 70°C for about 5 minutes and then cooled with continuous stirring. Formulation of ointment done by incorporating 10 g of the semisolid Ethanolic extract of *Thuja orientalis L.* into the various bases by triturating in a ceramic mortar with a pestle to obtain 100 g of herbal ointments containing 10 % w/w

of *Thuja orientalis L.* extract [33]. The prepared herbal ointments were put in ointment tube, labeled and were stored at room temperature.

2.2.6. Evaluation of ointment

The evaluations were carried out on the ointment by using the following parameters

Color and odour

Color and odour of ointment, examine by visual examination.

Loss on drying

1 g of ointment was placed in the Petridis and heated in the water bath at 105 °C every 30 min until it get constant weight.

pH

The pH of ointment was determined by digital pH meter. 1 g of ointment was dissolved in 50 ml of distilled water and the pH was measured.

Diffusion study

The diffusion study was carried out by preparing agar nutrient medium of any concentration. It was poured into Petridis. A hole bored at the centre and ointment was placed in it. The time taken for the ointment to get diffused was noted.

Stability study

The stability studies are carried out for the prepared ointment at temperature of 37°C for 2 months.

3. Results and discussion

The preliminary *in vitro* antimicrobial activity of the Ethanolic extract of *Thuja orientalis L.* (EETO) presented showed excellent activity against *Staphylococcus aureus*. The *in vitro* antimicrobial activity of the Ethanolic extract of *Thuja orientalis L.* (EETO) based herbal ointments. In various case history show that most of the infections are caused by the gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes*. Less common cause by the gram-negative bacteria such as *Escherichia coli*. The Ethanolic extract of *Thuja orientalis L.* (EETO) leaves showed significant antibacterial activity against all the tested microorganisms. This observation indicates that the activity due to the presence of large varieties of phytoconstituents present in the extract. Hence, the observed antibacterial activities of the ointment are due to the presence of active constituents of the extract and the activity also possess as ointment. This was good sign to do further studies on that to make it as one of the commercial ointment for the treatment of bacterial infections. In literature survey it was found that the plant possesses several traditional and pharmacological uses. The formulation and evaluation of herbal ointment study of the leaves extract of *Thuja orientalis L.* were done. The leaves were bright green in color. The values of the physical constant like loss on drying, Total ash value, Water soluble ash, Acid insoluble ash, Foreign matter, Swelling index, extractive value were determined. Extractive value and color of extract was investigated (Table-2).

Table 1 Macroscopical evaluation of *Thuja orientalis L* leaves

S. No.	Feature	Observation
1	Colour	Bright green
2	Odour	Characteristics
3	Taste	Strong camphoraceous
4	Size	Avg. 1-10 mm long

Table 2 Extractive value of *Thuja orientalis L.* Leaves

S. No.	Solvent	Wt. of Plant material (gm)	%age of yield	Colour of extract
1	Chloroform	4	5.1%	Greenish Brown
2	Pet. Ether	4	1.90%	Greenish yellow
3	Ethanol	4	5.8%	Greenish
4	Aqueous	4	4.7%	Dark green

Table 3 Physicochemical parameters of *Thuja orientalis L.* leaves

S. No	Physicochemical parameters	Observation
1	Loss of drying	8.82%
2	Total ash value	3.78%
3	Water soluble ash	0.95%
4	Acid insoluble ash	2.1%
5	Foreign matter	nil
6	Swelling index	1 cm

Table 4 Fluorescence Analysis of *Thuja orientalis L* leaves Powder

S. No	Treatment	Normal light	U.V. light (Short length)	U.V. light (long length)
1	Powder + HCL	GREENISH	BLUISH GREEN	DARK GREEN
2	Powder +10%KOH	GREENISH	GREENISH BLACK	BLACK GREEN
3	Powder +H2SO4	GREENISH	DARK GREEN	BLACKISH GREEN
4	Powder +iodine	BROWNISH	DARK BROWN	BROWNISH BLACK
5	Powder + picric acid	YELLOWISH	DARK GREEN	BLACKISH GREEN
6	Powder+ Fecl3	BROWNISH	BLACKISH BLUE	BLACKISH
7	Powder + glacial acetic acid	GREENISH	BROWNISH GREEN	BROWNISH BLACK

Table 5 Composition of the Ethanolic extract of *Thuja orientalis L.* leaves ointment

S. No.	Components	Amount(gm)
1	Ethanolic extract of leaves Plant <i>Thuja orientalis L.</i>	2
2	Emulsifying wax	28
3	White soft paraffin	50
4	Liquid paraffin	20

Table 6 Physicochemical parameters of *Thuja orientalis L.* herbal ointment formulation

S. No	Physicochemical parameters	Observation
1	Colour	Dark greenish
2	Odour and taste	Characteristic
3	Loss of drying	0.2%
4	pH	6.3
5	Diffusion study	2.1cm in 1 min
6	Stability study	Stable with pH 6.2

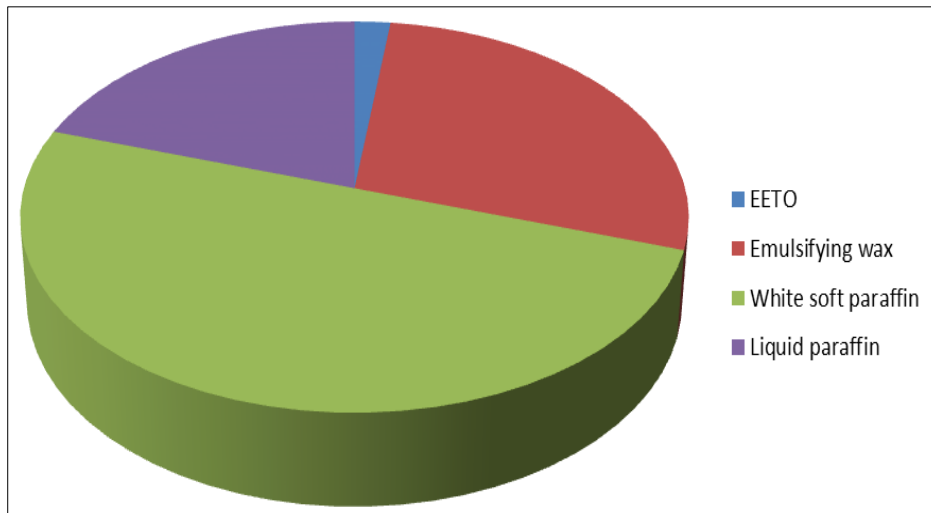


Figure 1 Composition of the Ethanolic extract of *Thuja orientalis L.* leaves ointment

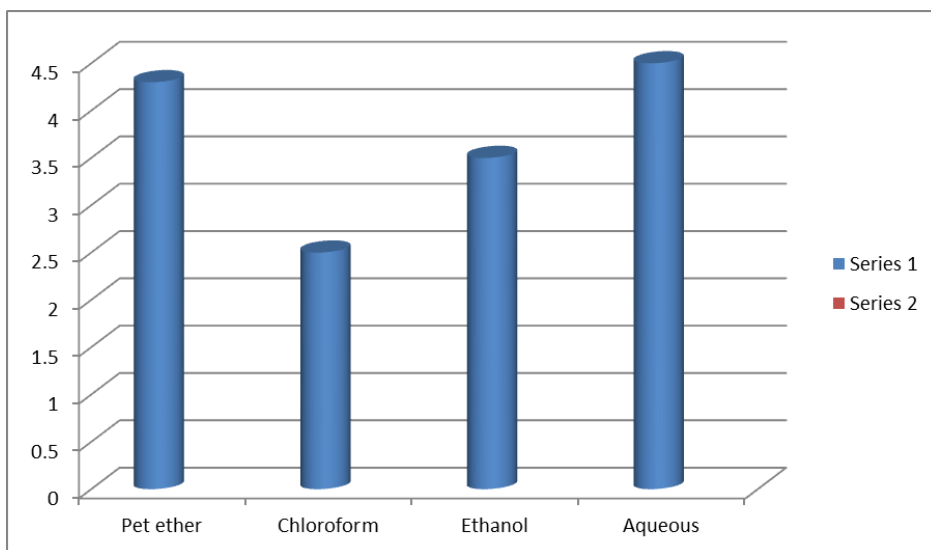


Figure 2 Extractive value of *Thuja orientalis L.* Leaves

Table 7 Zone of inhibition of the Ethanolic extract of *Thuja orientalis L.* (EETO)leaves

S. No	Concentration (mg/ml of EETO)	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
1	100	15.67 ± 3.05	15.00 ± 2.00
2	250	17.67 ± 1.53	17.00 ± 3.05
3	500	20.00 ± 3.00	18.33 ± 3.06
4	Ciprofloxacin (5 µg)	29.33 ± 3.06	27.00 ± 2.00

4. Conclusion

It is concluded that the present investigation comes out with the fact that *Thuja orientalis L.* essential oil are required so that better, safe and cost effective drugs for treating *S. aureus* causing diseases. This study shows that *Thuja orientalis L.* are antibacterial activity and have high potential as antibacterial agent. When formulated as ointment for topical use and could therefore explain the successes claimed in the folk use of the plant in the treatment of common skin conditions. The potency of the *Thuja orientalis L.* herbal ointment against *Staphylococcus aureus* could be harnessed in the containment of the organism implicated as the commonest etiologic agent of boils, carbuncles, infantile- impetigo and wound. The final product readily spread on skin surface, showed no irritant effect, diffused well and stable at different temperature. It also require to research on phytochemical and pharmacological aspect. However research going on it would be easier to develop new formulation.

Compliance with ethical standards

Acknowledgments

Authors express sincerely thanks to Dr. Emdad Hossain, principal, Pharmacy College, Azamgarh, and Uttar Pradesh for providing research facilities.

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