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Physico-Chemical Analysis and Phytochemical Evaluation of *Thuja occidentalis* Leaves

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ABSTRACT

The present research work deals with determination of physicochemical analysis and phytochemical investigation of *Thuja occidentalis* leaves. Standardization of crude drug extracted from plant plays an important role in identifying the quality and purity of drugs. The present research analysis reveals standardization of crude drug that includes moisture content, total ash, acid insoluble ash, water soluble ash, and different soluble extractive values were estimated. The highest extractive values were recorded in water soluble extract of crude drug, the bioactive principles present in medicinal plants and that may lead to drug discovery and development. In the present study deals with phytochemical constituents of the *Thuja occidentalis* medicinal plant of Cupressaceae family were identified in order to relate their presence with bioactivities of the plants. The present research highlights that methanolic extracts of *Thuja occidentalis* had the highest number of phytochemicals compared to other solvent extracts. Hence, methanolic extracts of *Thuja occidentalis* holds the greatest potential to treat various human diseases and has profound medical applicability.

INTRODUCTION

In many developing countries, major proportion of the population are depends on traditional practitioners and using medicinal plant products in order to get their health in good conditions[1].

The use of medicinal products and supplements has increased exceedingly over the past decades, not less than 80% of world population are depends on medicinal plant products for their primary health. *Thuja occidentalis*, known as white cedar, is indigenous to eastern North America and is grown in Europe as an ornamental tree [2]. The plant was first recognized as a remedy by native Indians in Canada during a 16th century expedition and was found to prove effective in the treatment of weakness from scurvy [3]. Getting knowledge about the chemical constituents of plant is essential for usage in medicine and also for the synthesis of complex chemical components [4]. An association between the phytoconstituents and their bioactivity of the plants are desirable to know for the synthesis of compounds with specific activities to treat various health issues and chronic diseases as well. *Thuja* leaves are utilized for the treatment of various diseases like respiratory tract infections such as bronchitis, bacterial skin infections, and cold sores [5]. It is also utilized for the treatment osteoarthritis and a nerve disease that affects the face called trigeminal neuralgia, also used for the treatment of Cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism [6,7]. Understanding about physicochemical and preliminary phytochemical screenings of plants is needed in order to discover and develop novel therapeutic agents with improved efficacy. A number of plant species that thrive in hostile environment replete with bacteria, fungi or virus synthesize defensive natural products against these pathogens, they may also exhibit bactericidal, fungicidal or virucidal activity in human beings [8-10].

Several scientists reported on antimicrobial activities of various medicinal plants and found that these plants are effective against the microorganisms which showed drug resistance to various antibiotics [11,12]. They were screened on numbers of medicinal plants for their antimicrobial activity and studied the effect of various plant extracts on bacteria producing diseases in human being [13-17]. The present research work deals with determination of physicochemical analysis and phytochemical screening of *Thuja occidentalis* leaves.

MATERIALS AND METHODS

Collection of the Plant Material

Thuja occidentalis leaves were collected from the college ground, Mother Teresa Pharmacy College, Sathupally, Khammam, Telangana.

Preparation of Thuja occidentalis leave powder

Plant leaves are collected and air dried because to prevent it from direct sunlight impact to minimize undesirable chemical reactions of plant metabolites. Dry conditions are crucial to prevent the formation of artifacts as a result of microbial fermentation and subsequent degradation of the plant metabolites. Hence in the present study, leaves were dried in shade and then powdered with a mechanical grinder. The powder was passed through sieve number 44 and stored in an airtight container for further studies.

Physicochemical Analysis

Physicochemical analysis includes moisture contents, ash values and extractive values were determined to study the quality and purity of the powder of *Thuja occidentalis* leaves.

Moisture Content

5g of the air-dried sample was weighed and it was noted as (W_b), into a pre-dried and weighed (W_a) soiled porcelain crucible. The sample was dried in a hot air oven at 100°C until two consecutive weighing's (W_c) do not vary by more than 5mg. The moisture content of the sample was deliberated with reference to crude air dried drug.

$$\text{Moisture (\%)} = \frac{(W_b - W_C)}{(W_b - W_a)} \text{ x } 100$$

Total Ash Value

A silica crucible was heated until it becomes redness and cooled in a desiccator and weighed (W_1). About 5g of air-dried sample was placed to the silica crucible and weighed along with the contents accurately (W_2). Sample was burst into flame gradually in an electrical muffle furnace, increasing the heat to 450°C until it is white, indicating the absence of carbon. It was cooled in desiccators and reweighed (W_3), and the total ash content was determined as in equation.

$$\text{Total ash (\%)} = \frac{(W_3 - W_1)}{(W_2 - W_1)} \times 100$$

Acid-insoluble Ash

To the silica crucible containing the total ash 10ml of 2*M* HCl were added, covered with a watch-glass and boiled lightly for 5min. The watch-glass was cleaned with 5ml of hot water and the washings were added to the crucible. The insoluble matter was filtered on an ash less filter-paper and cleaned with hot water until the filtrate is neutral. The filter-paper containing the insoluble content was kept into the original crucible, dried on a hotplate and ignited to constant weight (W_4). The residue was allowed to cool in desiccators for 30min, and then it was weighed. Acid-insoluble ash content was determined as in equation.

$$\text{Acid-insoluble ash (\%)} = \frac{(W_4 - W_1)}{(W_2 - W_1)} \times 100$$

Extractive Values

The extractive values of *Thuja occidentalis* leaves in various solvents like petroleum ether, benzene, chloroform, ethyl acetate, methanol, ethanol and water were estimated by employing the method of analysis described in Pharmacopoeia of India [18]. About 5g of air-dried *Thuja occidentalis* leave powder was taken in a stopper conical flask. 100ml of the respective solvent were mixed, shaken well and allowed to stand for 24h with occasional shaking. Then it was filtered, 50ml of the filtrate was pipetted out into a clean, previously weighed china dish and evaporated on a water bath. Lastly, it was dried at 100ºC in an oven, cooled in a desiccator and weighed. The percentage of solvent soluble extractive with reference to the air-dried content was calculated.

Water Soluble Ash

25ml of water was added to the crucible containing the total ash and boiled for 5min. The insoluble components were collected on an ash less filter-paper. The filter was cleaned with hot water and then ignited in a crucible for 15min at a temperature not exceeding 400°C. The residue was confessing to cool in desiccators for 30min, and then re-weighed (W_5), calculations were finished according to equations.

Weight of residue, W_6 (g) = W_5 - W_1Weight of ash W_7 (g) = W_3 - W_1Water-soluble ash (g) = W_7 - W_6

$$\text{Water-soluble ash (\%)} = \frac{(W_7 - W_6)}{(W_1)} \times 100$$

Preliminary Phytochemical ScreeningPreparation of Plant Extract

The coarse powder of the plant material was weighted (20g) and placed into the earthy colored glass bottles. The coarse powder was exposed to extraction in 250ml every one of petroleum ether, chloroform, and methanol solvents independently. At that point, the solvents were added to it. At that point, the containers were fixed with aluminum foil and kept in research center shaker at room temperature, and the flasks were shaken for 5 days. At last, the concentrate was sifted through numerous layers of muslin fabric for coarse filtration. The coarse filtrate was than separated through Whatman filter paper number 1. They got filtrate was vanished in a vacuum turning evaporator under decreased pressing factor at 40ºC until the filtrate was diminished to 33% of the beginning filtrate volume and the concentrated concentrates were additionally dissipated to get dry concentrates. A piece of dry concentrates were re-disintegrated in dimethyl sufloxide (DMSO) and were put away in plug glass bottles and another part was kept as such in hermetically sealed containers at 4ºC for additional examination.

Phytochemical Screening

The phytochemical screening establishes regarding the presence of different compounds possessing therapeutic effects. The different solvent extracts of *Thuja occidentalis* leaves were used for screening the presence of carbohydrate, glycosides, alkaloids, flavonoids, steroids, coumarin, tannins, saponins, phenol, protein, xanthoprotein, catachin, quinone, anthroquinone, sugar and terpenoids according to standard procedures [19-22].

Screening for Carbohydrates (Molisch Test)

3 drops of α-naphthol (20% in ethanol) were added to 2ml of extract sample. Then 1ml of concentrated sulphuric acid was added by side of the test tube. Reddish-violet ring was observed at the junction of the two layers indicated the presence of carbohydrates.

Screening for Glycosides (Borntrager's test)

50mg of extract powder was mixed with 5ml of concentrated H_2SO_4, it was heated for 5min, and it was filtered. The filtrate was mixed with 0.5ml of 10% NaOH and kept a side for 5min.

Production of reddish brown precipitate indicates the presence of glycosides.

Screening for Alkaloids (Dragendroff's test)

2ml of the extracted samples were mixed with 8ml of 1% HCl, heated and filtered. Then the filtrates were mixed with solution of Potassium Bismuth Iodide (Dragendroff's reagent). Red color precipitate was observed, indicates the presence of alkaloids.

Screening for Reducing Sugar

50mg of the extract powder wastaken in a test tube and equal volume of Fehling reagents (A and B) were added and boiled. Production of brick-red precipitate indicates the presence of reducing sugar.

Screening for Terpenoids (Salkowski test)

5ml of solvent extract was treated in 2ml of chloroform and then added of 3ml concentrated sulfuric acid (H_2SO_4). Production of reddish brown color was formed at the interface that indicates the presence of terpenoids.

Screening for Steroids (Liebermann Burchard test)

Extracted samples were treated with chloroform and filtered. The filtrates were mixed with few drops of acetic anhydride, heated and cooled. By addition of concentrated sulphuric acid, it produced brown ring at the junction indicates the presence of phytosterols.

Screening for Tannins

In 25ml distilled water, 50mg of various solvent extracted powder was placed and filtered. 1% aqueous ferric chloride ($FeCl_3$) solution was added to the filtrate. The appearance of different colors like green, purple, blue or black that indicate the presence of tannins in the test samples.

Screening for Saponin

In 25ml distilled water, 50mg of the various solvent extracted powders were boiled in boiling water bath and filtered. 10ml of the filtrate was treated with 5ml of distilled water and was shaken vigorously to the formation of stable persistent froth. The frothing was treated with 3 drops of olive oil and shaken vigorously for the production of emulsion thus a characteristic

of saponins.

Screening for Flavonoids (Shinoda Test)

To 5ml of the extracted solution, few fragments of magnesium ribbon and concentrated HCl were added dropwise. Production of red or orange red color indicates the presence of flavonoids.

Screening for Quinone

1ml of the extract was treated with 1ml of concentrated H_2SO_4; production of red color shows the presence of Quinone.

Screening for Anthroquinone (Borntrager's test)

50mg of extract powder sample was placed into a test tube, to this 5ml of chloroform added and shaken for 5min. The extract was filtered through Whatman No 1 filter paper and to the filtrate equal volume of 10%, ammonia solution was added and shaken for 5min. A pink violet or red color at lower layer of ammonia solution indicates the presence of anthroquinone.

Screening for Phenols

50mg of extract powder was dissolved in 5ml of distilled water. Few drops of 10% ferric chloride solution were added to this. Production of blue or green color indicates the presence of phenol compounds.

Screening for Protein

50mg of extract sample powder was dissolved in 10ml of distilled water and filtered through Whatman No. 1 filter paper. 1ml of 40% NaOH was added to the filtrate. Then, added 1 or 2 drops of 2% copper sulfate solution. Production of violet color indicates the presence of proteins.

RESULTS AND DISCUSSION

The physicochemical parameters are mainly used to get more information about the purity and quality of the drug. The extracted powder was evaluated for its physiochemical parameters like moisture content, total ash, water soluble ash, acid soluble ash, sulphated ash

and different solvent extractive values (Table 1). Moisture content is one of the major factors responsible for the degradation of drugs and herbal formulations. The moisture content promotes the degradation processes caused by enzymes, development of microorganisms, oxidation and hydrolysis reactions. The residues endure after incineration of plant material is known as total ash or ash value. A high ash value is symbolic of contamination, substitution or adulteration by mineral components. Ash value constitute both physiological ash and non-physiological ash, physiological ash is obtained from plant tissue due to biochemical processes while non-physiological ash consist of residue of the extraneous matter (sand, soil etc.) deliberately or non-deliberately adhering to plant samples. Physiological ash disintegrates in the dilute acid and non-physiological ash remains same. In the total ash content, water-soluble ash value is also part of it, which is soluble in water. This study shows 2.15 % water soluble ash is present in *Thuja occidentalis* leaves. In the total ash content, acid insoluble ash measures the amount of silica present especially as sand and siliceous earth in the samples. These values indicate the magnitude of presence of phosphates, oxalates, carbonates, oxides and silicates. Hence, these values are indices of excellence of herbal remedies.

Table No. 1: Physicochemical analysis of *Thuja occidentalis* leaves

Constants	**Percentage**
Moisture contents	8.22 ± 0.20
Total ash contents	0.60 ± 0.05
Acid soluble ash	1.40 ± 0.05
Water soluble ash	2.15 ± 0.05
Extractive values	
Methanol	2.5±0.03
Chloroform	1.5±0.05
Petroleum ether	2.4±0.02

Preliminary phytochemical screening of plants was predominant to the detection of bioactive principles which is a new source of therapeutically and industrially valuable compounds that may lead to the discovery of new drugs. In the present study, the presence of phytochemicals were screened with the petroleum ether, chloroform, and methanol extracts of the *Thuja occidentalis* leaves and the results are shown in Table 2. Crude extracts and medicines are manufactured based on the principles of natural compounds even by pharmaceutical

companies, may lead to large scale exposure of humans to natural products. Presence or absence of important bioactive compounds in extracts were identified by color reactions with specific chemicals, this procedure is simple for preliminary pre-requisite before going to phytochemical investigation. Hence, in the present work, the crude extracts obtained by petroleum ether, chloroform, and methanol solvents were screened for the presence of phytochemicals. The methanol extract shows the presence of steroids, saponins, flavonoids, phenols, proteins, glycosides and terpenoids. Saponins have health benefits such as lower cholesterol, antimicrobial, anti-inflammatory and anticancer properties[23]. Many herbal medicinal researches have established saponins as the active components and their contributions to the health benefits of foods such as soybeans and garlic.

Table No. 2: Preliminary phytochemical screening of *Thuja occidentalis* leaves

Test	Petroleum ether	Chloroform	Methanol
Alkaloids	-	+	+
Steroids	-	+	+
Tannins	-	-	-
Saponins	+	+	+
Phenols	+	-	+
Flavonoids	+	+	+
Terpenoids	-	+	+
Glycosides	-	+	-
Proteins	-	-	-

+ indicates the presence of the phytochemical;

- indicates the absence of the phytochemical

Phenolic compounds have biological and pharmacological properties such as anti-inflammatory, antioxidant, and antimutagenic and anticarcinogenic activities. Flavonoids are secondary metabolite having various pharmacological properties such as anti-oxidative, anti-fungal, anti-inflammatory and diuretic actions[24,25]. This research finding highlights that methanolic extracts of *Thuja occidentalis* leaves had the highest number of phytochemicals compared to other solvent extracts. Hence, methanolic extracts of *Thuja occidentalis* leaves holds great potential to treat various human diseases and has profound medical applicability.

CONCLUSION

The physicochemical parameters gave information about the purity and quality of the drug. The presence of phytoconstituents, such as phenols and flavonoids in plants, indicates the possibility of antioxidant activity and this activity will help in preventing a number of diseases through free radical scavenging activity. Since the plant *Thuja occidentalis* leaves has been used in the treatment of different ailments, the medicinal roles of this plant could be related to identify bioactive compounds. The present analyses suggest that *Thuja occidentalis* leaves contain potentially health-protective phytochemical compounds with a potent source of natural antioxidants and antibacterial activities that may be clinically promising.

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