



## PRELIMINARY PHYTOCHEMICAL ANALYSIS OF LEAF EXTRACTS OF THUJA ORIENTALIS (L.) ENDL

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### Abstract

The objective of the present study was to investigate the presence of various phytochemicals from the methanolic, ethanolic, aqueous and ethyl acetate extracts of leaf extracts of Thujaorientalis (L.) Endl. Preliminary phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, terpenoids, tannins, phenols, anthraquinone, glycosides, oils and fats, and carbohydrates. The present study provides evidence that solvent extracts of Thujaorientalis (L.) Endl contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

### Introduction

The therapeutic efficacy of many indigenous plants for various diseases has been described by traditional herbal medicinal practitioners. Medicinal plants are the source of synthetic and traditional herbal medicine [1]. The medicinal value of plants lies in some chemical active substances that produce a definite physiological action on the human body. Many of these are used as the active ingredients of the modern medicine, or as the lead compound for new drugs discovery.

Thujaorientalis (L.) Endl belonging to the family Cupressaceae is a tree of about 20-25 m height, and it is commonly called morpankhi. The plant has used in the different activities that are antipyretic, antitussive, astringent, diuretic, refrigerant and stomachic [2]. High percentage of polyphenolic content in the plant showed significant antioxidant and antimicrobial activity [3]. The phytoconstituents of T. orientalis have reported the treatments of gout, rheumatism, diarrhoea and chronic tracheitis [4]. The present work deals with phytochemical screening of T. orientalis leaves extract and various fractions.

### Materials and methods

#### Plant materials

Leaves of Thujaorientalis (L.) Endl were collected in Cu Chi District, Ho Chi Minh City, Viet Nam in October, 2014. The specimen was identified and deposited in the herbarium of Applied Biochemistry Laboratory, Department of Applied Chemistry, School of Biotechnology, International University, Viet Nam National University-Ho Chi Minh City.

#### Preparation of extracts

Fresh leaves of Thujaorientalis (L.) Endl were dried in and ground into fine powder and referred to as powdered leaves. 20g portions of powdered plant materials were each separately dissolved in 300 ml of each methanol, ethanol, distilled water and ethyl acetate. The solution was left to stand at room temperature for 24hrs with occasional shaking and was filtered with Whatman No. 1 filter paper. The filtrate was used for the phytochemical screening using the following tests.

#### Photochemical screening of extract

Phytochemical analysis was carried out for all the extracts according to the standard methods [5,6, 7,8,9].

#### Test for alkaloids

Small quantities (2ml) of various extracts were separately boiled with 5 ml of 2% hydrochloric acid on a water bath for 5 minutes. The mixture was allowed to cool and filtered. The filtrates were used for the following test.

**Wagner's test:** Filtrates were treated with few drops of Wagner's reagent. Formation of brown reddish precipitate indicates the presence of alkaloids.

**Dragendroff's test:** Filtrates were treated with Dragendroff's reagent; presence of alkaloids confirmed by the formation of red precipitate.

**Hager's test:** Filtrates were treated with Hager's reagent. Appearance of yellow colored precipitate indicated presence of alkaloids

#### Test for flavonoids

A small quantity of various extracts was heated with 10 ml of ethyl acetate in water bath for 3 minutes. The mixture is filtered differently and the filtrates are used for the ammonium and aluminum chloride test.



**Ammonium test:** 4ml of the filtrate was shaken with 1 ml of 1% ammonia solution. The layers were allowed to separate. A yellow color was observed at ammonia layer. This indicates the presence of the flavonoid.

**Aluminum chloride test:** 4ml of the filtrates were shaken with 1 ml of 1% aluminum chloride solution and observed for light yellow color. It indicated the presence of flavonoid and diluted sodium hydroxide and hydrochloric acid was added. A yellow solution that turns colorless indicated presence of flavonoid.

**Ferric chloride test:** small quantities of various extracts was dissolved in 2ml ethanol, few drops of 10% ferric chloride were added. The formation of green- blue coloration confirmed the presence of flavonoids.

**Lead acetate test:** 1ml of extracts was treated with few drops of 10% lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

### Test for saponins

**Frothing test:** Small quantities of different extracts were diluted with 4 ml of distilled water. The mixture was shaken vigorously and then observed on standing for stable froth.

### Test for tannins

Small quantities of the various extracts were taken separately in 20ml of distilled water, boiled, filtered and test for the presence of tannins was carried out with the following reagents.

**Ferric chloride test:** To 2ml of the filtrate, few drops of 5% ferric chloride were added. Formation of a greenish black precipitate indicated the presence of tannins.

**Lead acetate test:** To 2ml of the filtrate was added few drops of 10% lead acetate solution. Formation of precipitate indicated the presence of tannins.

### Test for phenolic compounds

**Ferric chloride test:** 1ml of different extracts was treated with 3-4 drops of 10% ferric chloride solution. Formation of deep blue or black color indicates the presence of phenols.

### Test for steroids

**Lieberman-burchard's test:** 2ml of the solvent extracts were dissolved in 2ml of chloroform to which 10 drops of acetic acid and five drops of concentrated sulphuric acid were added and mixed. The change of red color through blue to green indicated the presence of steroids.

### Test for terpenoids

**Salkowski Test:** 5 ml of various solvent extract was mixed in 2 ml of chloroform followed by the careful addition of 3 ml concentrated sulphuric acid to form a layer. A layer of the reddish brown coloration was formed at the interface thus indicating a positive result for the presence of terpenoids.

### Test for carbohydrates

A small quantity of various extracts were dissolved separately in 4ml of distilled water, boiled and filtered. The filtrate was subjected to the following test to detect the presence of carbohydrates.

**Fehling's test:** About 2 ml of the filtrate was added to about 2 ml of Fehling's solution taken in a test-tube. It is then boiled for 10 min. A brick red precipitate demonstrated the positive result for carbohydrates.

### Test for glycosides

**Modified boratrager's test:** 5 ml of each extract was added 5 ml of 5% ferric chloride solution and 5 ml dil. hydrochloric acid, heated for 5 minutes in water bath. Cool and add 3ml of benzene or organic solvent. Shake well. Separate organic layer, add equal volume of 10% ammonia solution. The formation of rose pink/red at ammonia layer shows the presence of glycosides.

### Test for anthraquinone glycosides

**Boratrager's test:** Added 1ml of dilute Sulphuric acid to 1ml of each extract. Boiled 10 min and filtered. The filtrate was shaken with 1ml of chloroform. Separated the lower layer of chloroform and shaken it with half of its volume of 10% ammonia solution. The formation of rose pink/red at ammonia layer showed the presence of glycosides.

### Test for phlobatannins

About 2 ml of different extracts was added to 2 ml of 1% hydrochloric acid and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

**Test for oils and fat**

**Sudan test:** 1ml of each extract was treated with a few drops of Sudan III solution. A shining orange color obtained showed the presence of fixed oil and fat.

**Saponification test:** Few drops of 0.5 N alcoholic potassium hydroxide was added to 1ml of various extracts along with drop of phenolphthalein, the mixture was heated 2 hours. Formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

**Test for protein**

**Biuret test:** A quantity each of extracts was put in a test-tube and treated with 5 drops of 1% hydrated copper sulphate. To this 2ml of 40% sodium hydroxide was also added and the test-tube shaken vigorously to mix the contents. A purple color showed the presence of proteins.

**Test for anthocyanin**

**NaOH Test:** 2ml of different extracts was treated with 2ml of NaOH. The presence of blue green color indicated the presence of anthocyanin.

**Result and discussion**

The result of the phytochemical analysis of four solvent extracts (methanol, ethanol, aqueous, and ethyl acetate) of *Thujaorientalis* (L.) Endlis summarized in Table 1.

*Table 1: Preliminary Phytochemical Analysis of Thujaorientalis (L.) Endl*

No	Phytochemical test		Extracts			
	Chemical constituents	Tests	Methanol	Ethanol	Aqueous	Ethyl Acetate
1	Alkaloids	Wagner's test, Dragendroff's test, Hager's test	+	+	-	+
2	Flavonoids	Ammonium test, Aluminum Chloride test, Lead acetate test, Ferric Chloride test	+	+	+	-
3	Saponins	Frothing test	-	-	-	-
4	Tannins	Ferric Chloride test, Lead acetate test	+	+	-	-
5	Phenolic compounds	Ferric Chloride test	+	+	+	+
6	Steroids	Lieberman-Burchard's Test	-	-	-	-
7	Terpenoids	Salkowski Test	+	+	+	+
8	Carbohydrates	Fehling's test	+	+	+	+
9	Glycosides	Modified Borntrager's test	+	+	+	+
10	Anthraquinone	Borntrager's test	+	+	+	-
11	Phlobatannins	hydrochloric acid test	-	-	-	-
12	Oils and Fats	Sudan test, Saponification test	+	+	+	+
13	Protein	Biuret test	-	-	-	-
14	Anthocyanin	NaOH test	-	-	-	-

\*+ indicates positive test result; - indicates negative test result



Phenolic compounds, terpenoids, carbohydrates, oil and fat, and glycosides were present in all the extract whereas saponins, steroids, protein, phlobatannins and anthocyanin were completely absent in all the four solvent extracts of leaves of *Thujaorientalis* (L.) Endl as shown in Table 1. Except for ethyl acetate, flavonoids and anthraquinone were observed in the other three solvent extracts of *Thujaorientalis* (L.) Endl. Among the four solvent extracts of the leaves of *Thujaorientalis* (L.) Endl, tannins were observed in methanol and ethanol, but the presence of alkaloids was detected only in methanol and ethyl acetate extracts. The use of four solvents for extraction has revealed that the phytochemical composition of the extract varies with the solvent used [16, 17]. The phytochemical analysis in the present study has revealed the presence of alkaloids, flavonoids, tannins, phenolic compound, terpenoids, oil and fat, anthraquinone, carbohydrate and glycosides (Table 1) in the leaves extract of the study plant. Further the presence of chemical constituents in the four different extracts may be responsible for the therapeutic properties of *Thujaorientalis* (L.) Endl.

Flavonoids are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers [10,11]. Flavonoids are also shown to have antibacterial activity [12]. Since the compound was found to be present in the extracts, it might be responsible for the potent antioxidant capacity and antibacterial activity of *Thujaorientalis* (L.) Endl. The other chemical constituents of medicinal plant account for their medicinal value. For example, Glycosides are naturally cardio-active drugs used in the treatment of congestive heart failure and cardiac arrhythmia, so the presence of glycosides in the extracts might play a role in the cardioprotective potential of *Thujaorientalis* (L.) Endl [13]. Tannins are shown to have antiviral, antitumour, wound healing and antiparasitic effects [14]. Alkaloids contained in plants are used in medicine as anaesthetic agents [15].

The yield of bioactive metabolites in a plant extract also varies considerably with the solvent of extraction [16, 17], it is plausible that the methanolic extracts were generally more potent than the aqueous extracts probably because the active principles in the plant dissolved more readily in methanol and were better extracted by a less polar solvent (methanol, ethanol) than water. This is in agreement with many literatures reporting of differences in the activities of extracts obtained from the same morphological part of a plant using different solvents [18]. The results obtained in this study are helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compound.

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### Conclusion

The plants studied here can be seen as a potential source for treating different ailments. Further studies on the quantitative analysis of the various bioactive compounds present in *Thujaorientalis* (L.) Endl should be investigated to obtain useful drugs.

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