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(RESEARCH ARTICLE)



## Antimicrobial, antioxidant and phytochemical investigation of *Thuja occidentalis* (Arbor vitae) leave extract

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### Abstract

Various medicinal plants are common in use in India for the treatment of different diseases. Nowadays, drug resistance has emerged as a major problem for various infections, in such case plants can be used as alternative for the production of new medicine. *Thuja occidentalis* (*T. occidentalis*, Cupressaceae) is a well-known medicinal plant. The aim of the present study was to evaluate qualitative and quantitative phytochemical analysis and in vitro antioxidant and antimicrobial activities of leaf of *T. occidentalis* collected from Bhopal region of Madhya Pradesh. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folin Ciocalteu reagent method and aluminium chloride method respectively. The *in vitro* antioxidant activity of methanolic extract of the leaves was assessed against DPPH and reducing power assay method using standard protocols and in vitro antimicrobial activity was done by well diffusion assay method. Phytochemical analysis revealed the presence of alkaloids, glycosides, phenols, flavonoids, tannins. The total phenolics content of leaves of methanolic extract was (0.125 mg/gm), followed by flavonoids (0.475mg/gm). The activities of all leaves extract against DPPH and reducing power assay method were concentration dependent. The antimicrobial acuity of methanolic extract of leaf against all microorganisms was concentration dependent manner but less than standard drug (Ofloxacin, Amphotericin B). The present study concluded that the crude extract of *T. occidentalis* is a rich source of secondary phytoconstituents which impart significant antioxidant and antimicrobial potential. The findings of the present study will be helpful to phytochemists, pharmacologists and pharmaceutical industries.

**Keywords:** *Thuja occidentalis*; Physicochemical; Qualitative; Quantitative phytochemical; Antioxidant; Antimicrobial.

### 1. Introduction

Various plants have been used as traditional medicine from the ancient time of human civilization. Many ancient documents revealed that plants were used for medicinal purposes in china, India, Egypt and Greece long before the beginning of Christian era. Various plants also contain antioxidants, which help in shielding the cells against the harmful effects of reactive oxygen species. Antioxidants are present in our body in an appropriate quantity but to protect our body from these reactive oxygen species (ROS) there is need of antioxidants from natural so urces [2]. The medicinal plants possess phenolic compounds that possess very strong antioxidant activity and in turn help the body against the damage by free radicals [3]. These phenolic compounds act as radical scavengers, reducing agents, nascent oxygen quenchers, hydrogen donors and metal chelators [4]. An antioxidant is defined as any substance which when present at

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low concentrations significantly delays or prevents oxidation of an oxidizable substrate by scavenging free radicals [5]. Free radicals and other reactive oxygen species are formed constantly in the human body. They can be useful for some physiological activities but in excess, they may prove very dangerous and harmful for human body. These free radicals generally damage the DNA, carbohydrates, lipids and proteins in body. Free radicals are also produced by environmental pollutants, cigarette smoke, automobile exhaust fumes, radiation, air pollutants and pesticides etc. [6]. There is a balance between the free radicals in body and the antioxidants to scavenge these radicals to protect against their harmful effect. However any increase in free radicals inside the body can produce harmful effects and stress in human body. Thus antioxidants are useful for maintaining the required quantity of free radicals inside the body and scavenge the extra free radicals so that no deleterious effects of these free radicals are found in human body. Plants also used as antimicrobials before microbiological study had even been started [7]. Plant derived bioactive substances are considered a very good and cheap source of medicines that play a significant role for human health improvement as well as used against different types of microbial disease [8,9]. Plants have great medicinal relevance because infections caused by drug resistant microorganisms have become a major therapeutic problem nowadays [10]. In addition, plant extract and their phytochemicals are getting more importance as they have the great potential sources for microbial and viral inhibitors. Therefore, thousands of researchers have focused their interest to investigate phytochemical constituents of plant for human health [11]. The bioactive constituents of plants such as tannins, flavonoids, saponins, terpenoids and alkaloids have great antimicrobial activity [12]. *T. occidentalis*, commonly known as Arbor vitae or white cedar, is indigenous to eastern North America and is grown in Europe as an ornamental tree [13]. The plant was first identified 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 [14]. In folk medicine, *T. occidentalis* has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism [15-18]. Today, it is mainly used in homeopathy as mother tincture or dilution [19, 20]. In combination with other immunomodulating plants, such as *Echinacea purpurea*, *Echinacea pallida* and *Baptisia tinctoria*, this medicinal plant is also used as evidence-based phytotherapy for acute and chronic infections of the upper respiratory tract [21, 22], and as an adjuvant to antibiotics in severe bacterial infections such as bronchitis, angina, pharyngitis, otitis media and sinusitis [23,24]. Therefore this study was conducted with the aim to investigate phytoconstituents present in leaves of *T. occidentalis* and assessment of their antioxidant and antimicrobial characteristics.

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## 2. Material and methods

### 2.1. Plant material

The leaves of *T. occidentalis* were collected from local area of Bhopal (M.P.) in the month of February, 2018. The sample was identified by senior Botanist Dr. Zia-Ul-Hassan, Professor and head of department of Botany, Safia College of Arts and Science, peer gate Bhopal. A herbarium of plants was submitted to the specimen library of Safia College of Arts and Science, peer gate Bhopal and The specimen voucher no. of *T. occidentalis* is 119/Bot/Saf/45.

### 2.2. Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

### 2.3. Bacterial strain

The test organisms *Pseudomonas auregenosa* (MTCC 2075), *Bacillus subtilis* (MTCC 1133), *Salmonella* (MTCC 1169), *Bacillus cereus* (MTCC 1305), *Aspergillus niger* (MTCC 872), *Candida krusei* (MTCC 9215) were obtained from the stocks of the Pinnacle Biomedical Research Institute, Bharat Scout & Guides Campus, Shyamla Hills, Bhopal, (M.P.).

### 2.4. Maintenance of bacterial culture

The Bacterial strains were grown on nutrient agar media and inoculate at 37°C temperature for 24 hours and fungal strains were grown on SDA medium. Then organisms were maintained on nutrient agar slant at 4°C temperature and the purity of the organisms was checked at regular intervals by plating.

### 2.5. Extraction of plant material

Leaves of *T. occidentalis* were collected, washed and rinsed properly. They were dried in shade and powdered mechanically. About 500gm of the leaves powder was macerated with methanol and stored for 72 hours in ice cold condition for the extraction of phytochemicals. At the end of the third day extract was filtered using whatman No. 1 filter paper to remove all un-extractable matter, including cellular materials and other constitutions that are insoluble in the

extraction solvent. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts [25].

## 2.6. Qualitative phytochemical analysis of plant extract

The *T. occidentalis* leaves extract obtained was subjected to the preliminary phytochemical analysis following standard methods [26, 27]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

## 2.7. Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC and TFC are determined. Extracts obtained from leaves of *T. occidentalis* plant material of subjected to estimate the presence of TPC and TFC by standard procedure.

### 2.7.1. Total phenolic content estimation

The amount of total phenolic in extracts was determined with the folin ciocalteu reagent. Concentration of (20-100 µg/ml) of gallic acid was prepared in methanol. Concentration of 100 µg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2 ml of a 10 fold dilute folin Ciocalteu reagent and 4 ml of 7.5% sodium carbonate. The tubes were covered with parafilm and it was then Incubated at room temperature for 30 mins with intermittent shaking and the absorbance were taken at 765 nm against using methanol as blank. Total phenolic content was calculated by the standard regression curve of Gallic acid and the results were expressed as gallic acid equivalent (mg/g) [28].

### 2.7.2. Total flavonoid content estimation

Different concentration of rutin (20 to 100µg/ml) was prepared in methanol. Test sample of near about same polarity (100µg/ml) were prepared. An aliquot 0.5ml of diluted sample was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a 5% NaNO<sub>2</sub> solution. After 6 min, 0.15 ml of a 10% AlCl<sub>3</sub> solution was added and allowed to stand for 5min, and then 2 ml of 4% NaOH solution was added to the mixture. The final volume was adjusted to 5ml with distilled water and allowed to stand for another 15 mins. Absorbance was determined at 510 nm against water as blank. Total flavonoid content was calculated by the Standard regression curve of Rutin/ Quercetin [29].

## 2.8. Antioxidant activity

### 2.8.1. DPPH radical scavenging activity

For DPPH assay, the method of Gulçin *et al.*, 2006 [30] was adopted. A solution of 0.1mM DPPH (4mg/100ml) in methanol was prepared and 1 ml of this solution was mixed with 1 ml of different concentrations of the different extracts. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. Ascorbic acid was used as reference standard while methanol was used as control. Reduction of the stable DPPH radical was used as a marker of antioxidant capacity of *T. occidentalis* extracts. The change in colour was measured at 517 nm wavelength using methanolic solution as a reference solution. This was related to the absorbance of the control without the plant extracts. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. All the tests were carried out in triplicates. Though the activity is expressed as 50% inhibitory concentration (IC<sub>50</sub>), IC<sub>50</sub> was calculated based on the percentage of DPPH radicals scavenged. The lower the IC<sub>50</sub> value, the higher is the antioxidant activity.

### 2.8.2. Reducing power assay

A spectrophotometric method was used for the measurement of reducing power. For this 0.5 ml of each of the extracts was mixed with 0.5ml phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of 1% potassium ferricyanide (10 mg/ml). The reaction mixture was incubated at 50°C for 20 min separately, and then rapidly cooled, mixed with 1.5 ml of 10% trichloroacetic acid and centrifuged at 6500 rpm for 10 min. An aliquot (0.5ml) of the supernatant was diluted with distilled water (0.5ml) and then ferric chloride (0.5ml, 0.1%) was added and allowed to stand for 10 min. the absorbance was read spectrophotometrically at 700 nm. Ascorbic acid (AA) was used as standard for construction of calibration curve [31].

$$\text{Reducing Power (\%)} = (As / Ac) \times 100$$

Here,  $A_c$  is the absorbance of control (AA) and  $A_s$  is the absorbance of samples (extracts) or standards.

## 2.9. *In vitro* antimicrobial activity

### 2.9.1. Well diffusion method

The agar well diffusion method technique Bauer *et al.*, 1966 [32] was used to determine the antibacterial activity of the plant extracts. Inoculation was done on sterile nutrient agar media plate. A sterile 5mm cork borer was used to punch holes after solidification of media. The wells formed were filled with different concentrations of the extract which were labeled accordingly; 100mg/ml, 150mg/ml, 200mg/ml, 250mg/ml. The plates were then left on the bench for 1 hour for adequate diffusion of the extracts and incubated at 37°C for 48 hours in upright condition. The Experiment was repeated triplets and the mean values were calculated.

### 2.9.2. Interpretation of well diffusion assay

After incubation, the diameter of the zones of inhibition around each well were measured to the nearest millimeters along two axis i.e. 90° to each other and the mean of the four reading were then calculated included 5mm well.

## 3. Results and discussion

The crude extracts so obtained after each of the successive cold maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The percentage yield of extraction is very important in phytochemical extraction in order to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from the leaves of the plants using methanol as solvents are depicted in the Table 1. The results of qualitative phytochemical analysis of the crude powder of leaves of *T. occidentalis* are shown in Table 2. Methanolic extracts of leaves sample of *T. occidentalis* showed the presence of carbohydrates, alkaloids, flavonoids, phenols, tannins and glycosides. Quantitative phytochemical assay was performed by calculating total phenolic content (TPC) and total flavonoid content (TFC). The TPC was calculated with respect to gallic acid (standard) and TFC was then calculated with respect to rutin taken as standard. The TPC and TFC in methanolic extract were found to be 0.125 mg/gm and 0.475 mg/gm respectively Table 3& Figure 1, 2. Antioxidant activity of the samples was calculated through DPPH assay and reducing power assay. % inhibition was calculated as an indicative of antioxidant potency. The higher the % inhibition the better the activity. Ascorbic acid was taken as standard in both the tests and the values were comparable with concentration ranging from 20µg/ml to 100µg/ml. A dose dependent activity with respect to concentration was observed. % inhibition was higher in the ascorbic acid while the values were lesser in methanolic extract Table 4. The reducing ability of the compound usually depends on the reductants, which have been exhibited antioxidative capacity by breaking the free radical chain, donating a hydrogen atom. Reducing power assay was calculated in extracts and the values indicated a better activity Table 5. *In-vitro* antimicrobial activity was also calculated in order to estimate the antimicrobial activity of the extracts. The method chosen to analyse the antimicrobial activity was well diffusion assay. Methanolic extract was tested for its antimicrobial activity on the organism's *B. cereus*, *Pseudomonas*, *Salmonella*, *B. subtilis*, *A. niger*, *C. albicans*. Varying concentrations of the extract was administered to the organisms consisting of 100, 150, 200 and 250 mg/ml. The zone of inhibition of all treated microorganisms was calculated. The values of zone of inhibition obtained were dose dependent, that is, the higher the dose the larger the zone of inhibition Table 6 & 7.

**Table 1** Results of percentage yield of leaves extracts

Plant Name	Percentage yield (%)
	Methanol
<i>T. occidentalis</i>	6.35

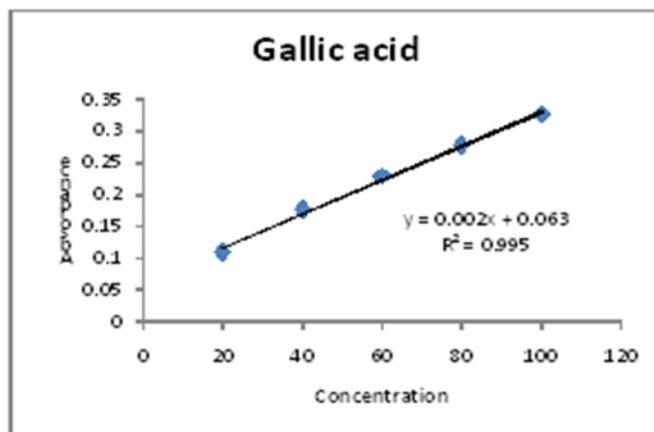
**Table 2** Phytochemical evaluation of *B. lanzan* leaves

Test	Methanolic extract
Carbohydrates	
Molish	+Ve
Fehling's	-Ve
Benedict's	-Ve
Test for protein and amino acid	
Biuret	-Ve
Ninhydrin	-Ve
Test for glycosides	
Borntrager's	-Ve
Keller-killani	-Ve
Test for alkaloids	
Mayer's	-Ve
Hager's	+Ve
Wagner's	+Ve
Test for saponins	
Froth Test	+Ve
Test for flavonoids	
Lead acetate	+Ve
Alkaline reagent	+Ve
Test for triterpenoids and steroids	
Salkowski's	+Ve
Liebermann-burchard's	+Ve
Test for Tannin and phenolic compounds	
Ferric chloride	-Ve
Lead acetate	+Ve
Gelatin	+Ve

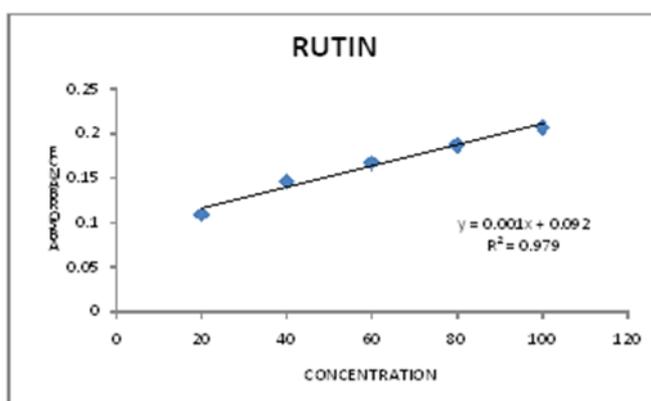
(+) Indicates Presence; (-) Indicates Absence

**Table 3** Total phenolic and flavonoid content of extracts

Test	Methanolic extract
TPC	0.125 mg/gm equivalent to Gallic acid
TFC	0.475 mg/gm equivalent to Rutin



**Figure 1** Graph of estimation of total phenolic content



**Figure 2** Graph of estimation of total flavonoids content

**Table 4** DPPH assay of ascorbic acid and methanolic extract

S. No.	Conc. (µg/ml)	Ascorbic acid (% Inhibition)	Methanolic Extract (% Inhibition)
1.	20	47.28	38.37
2.	40	54.17	45.06
3.	60	60.69	48.68
4.	80	68.84	55.81
5.	100	73.91	59.32
IC 50 Value		25.07	62.129

**Table 5** Result of reducing power assay

S. No.	Ascorbic acid	Methanolic Extract
1.	0.987	0.054
2.	1.032	0.14
3.	1.145	0.174
4.	1.159	0.188
5.	1.196	0.246

**Table 6** Antimicrobial activity of methanolic extract

<b>Extract</b>				
Organisms	100 mg/ml	150 mg/ml	200 mg/ml	250 mg/ml
<i>P. auregenosa</i>	7.75±0.500	9.00±0.816	10.00±0.000	12.00±0.816
<i>B. subtilis</i>	7.75±0.957	9.75±0.957	11.25±0.500	13.75±0.500
<i>Salmonella</i>	5.50±0.577	7.75±0.957	9.50±0.577	12.00±0.816
<i>B. cereus</i>	6.50±0.577	8.75±0.500	10.50±0.577	12.00±0.816
<i>A. niger</i>	8.50±0.577	10.25±0.500	11.75±75±0.500	13.50±0.577
<i>C. krusie</i>	8.50±0.577	10.75±0.500	11.75±0.500	12.50±0.577

**Table 7** Antimicrobial activity of Standard drugs

<b>Standard</b>					
<b>Ofloxacin</b>				<b>Amphotericin B</b>	
<i>P. auregenosa</i>	<i>B. subtilis</i>	<i>Salmonella</i>	<i>B. cereus</i>	<i>A. niger</i>	<i>C. krusie</i>
29.25±0.500	28.50±0.577	29.50±0.577	28.75±0.500	29.25±0.957	31.00±0.816

#### 4. Conclusion

*T. occidentalis* exhibited the potential antibacterial and antioxidant activities. The leaf extracts of this plant have various phytochemicals such as alkaloids, glycosides, flavonoids, steroids, tannins, terpenoids and saponins. Which are responsible for these activities? The methanolic extract has good antimicrobial activity against the tested microorganism. The plant exhibited the admirable antioxidant activity in methanol extracts. The observed good antioxidant activity of the extract indicates the potential of the leaves as a source of natural antioxidant.

#### Compliance with ethical standards

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##### Disclosure of conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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