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Evaluation of immunomodulatory and microbicidal potential of *Thuja occidentalis*

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Abstract

The present paper deals with the investigation for *in vitro* antimicrobial properties and immunomodulatory potential of *Thuja occidentalis*. Its various solvent extracts showed high potency of antimicrobial activities against bacterial pathogens. The growth of the bacteria *Escherichia coli* was found to be inhibited by all plant extracts. *Staphylococcus aureus* was found to be resistant to Hexane extract. While Ethanol extract inhibited the growth of *Staphylococcus aureus*. *Salmonella typhimuruim* cultures were inhibited by all extract. Water extracts were more effective against microbial cultures at high concentrations. Rapid rise in total WBC counts in treated organisms proved the plant to have high immunomodulatory property. Plant methanolic extract treated animals showed increased life span than untreated controls. ANOVA analysis of the results showed that the results were significant and reproducible.

Keyword: *Gastrointestinal pathogens*, *Humoral antibody response*, *WBC*, *Thuza occidentalis*

Introduction

Thuja occidentalis L. is indigenous to North America. It has coniferous pyramidal features, with flattened branches and twigs in one plane, bearing small scale-like leaves (British Herbal Pharmacopoeia, 1983). Over the whole year, the leaves are green, with the lower side showing a bright green colour where the resin glands reside. Small, 1–2 cm long green to brown coniferous pines contain the seeds. *Thuja occidentalis* is being used in several indigenous preparations for general health and other diseased condition. Plant metabolites possessing antimicrobial and immunomodulatory properties have been the significant part of traditional medicines (Mathew and Kuttan, 1999; Latha *et al*., 2000; Belal *et al*., 2005). Thus, in recent years there has been a high increase in the interest of researchers to explore the medicinal potential of plants (Chah *et al*., 2006; Madhuri and Govind, 2009). The present paper deals with the objective of evaluating the immunomodulatory and antimicrobial properties of *Thuja occidentalis* in the present study.

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Materials and Method

Thuja occidentalis plants were collected from Chitrakoot region, Uttar Pradesh. Leaves were dried for two days in the hot air oven at 37°C and then grinded in the grinder for three times. Powdered dry plant material was dissolved in different solvents and extracted using Soxlet assembly. Each extract was passed through Whatman Filter Paper No. 1. The extracts were concentrated by using vacuum evaporator at 32^0C and stored at 4^0C for further use. Antimicrobial activity was assayed by using broth dilution method (McKane and Kadel, 1986; Zahra *et al*., 2000; Coopoosamy and Magwa, 2007). Broth dilution test was used to determine the minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) of plant extracts as well as of standard drugs (Nair and Chanda, 2007).

The whole plants methanolic extract was used to study immunomodulatory properties. For the preparation of the extracts, dried ground plant material was percolated with 95 % methanol and concentrated to dryness under reduced pressure. Extract was redissolved in Dimethylsulphoxide (DMSO) to form stock solutions and their aliquots, which were filter sterilized (0.2 µm) before testing on cell lines. The sample was prepared in double distilled water along with 0.1 % acacia gum. Experimental Swiss mice (weighing 25±3 gm) were obtained from the Central Drug Research Institute (CDRI), Lucknow. The animals were housed in standard environmental conditions.

Preparation of Sheep red blood cells (SRBC) antigen

SRBC were collected aseptically from Jugular vein of sheep, stored in cold sterile Alsever's solution for immunization and challenge, at required time schedule. Stored sheep blood cells were centrifuged and washed three times with pyogen free sterile normal saline (0.85 % NaCl w/v) and adjusted to a required concentration for immunization (Nelson and Mildenhall, 1967).

Humoral antibody response (Hab) and Delayed type hypersensitivity (DTH-CMI)

Experiments were carried out in different groups (6 mice in each group) bearing cancer. On day 0 the mice were immunized by injecting 0.2 ml of 5×10^9 SRBC ml^{-1} ip and plant extracts were administered orally (100 mg/kg body wt.) for 5 consequent days after immunization. Two parallel controls were run simultaneously. One of them received only normal saline water which was named 'Normal Control', while the other received Levamisole (2.5 mg/kg body wt.) and Cyclo-phosphamide (250mg/kg body wt. post oral). Blood samples were collected from individual mice by retro-orbital puncture on day +5. Serial two fold dilution of 50 µl of serum sample of an individual mice was done in 50µl of normal saline containing 0.1 % BSA and added 50 µl of 0.1% suspension of SRBC in BSA. After mixing, the erythrocytes were allowed to settle at room temperature for about 60 to 90 minutes. The value of highest serum dilution causing visible haemagglutination was taken as the titre. The mean titre values of the drug treated groups were compared with the normal control. Doherty's (1981) method was employed to access SRBC induced DTH response in mice. The challenging dose of 20 µl of 5×10 SRBC/ml in mice were injected to assess the standard control response for DTH.

Calculation for immune response

Immunomodulatory activity = {(Test group - Control)/ Control} x 100

WBC Count

Swiss mice (n=6) bearing cancer with EAC were treated daily with *Thuja occidentalis* extract (100 mg/kg) ip for 5 days. Blood was collected by puncturing the retro-orbital plexus. Total WBC and RBC count was determined using a haemocytometer. A normal control group received normal saline (5 mg/kg/ip) and positive control group treated with 5-Fluorouracil (5- FU), an anticancer drug.

Results and Discussion

The results obtained were very interesting shown in Figure.1. Results were repeated thrice and significance of the data was analyzed using appropriate statistical packages. The growth of *Escherichia coli* bacteria was found to be inhibited by all extracts in comparison to control (O.D. 1.56), but ethanol extract (O.D. 1.34) showed more inhibition than other extracts. Water extract (O.D. 1.42) and hexane extract (O.D. 1.43) showed nearly same inhibitory effect. Chloroform extract (O.D. 1.48) showed least inhibitory effect. In the case of *Staphylococcus aureus,* the cultures were found resistant to hexane extract (O.D. 1.83) with respect to control (O.D. 1.83). Chloroform extract (O.D. 1.77) showed a little inhibition of cultures. While ethanol extract (O.D. 1.71) was found inhibiting the growth of *Staphylococcus aureus*. Water extract (O.D. 1.85) had no effect on it. In the case of *Pseudomonas aeruginosa*, water extract (O.D. 1.79) showed resistance towards these bacteria in comparison with control. Ethanol extract (O.D. 1.35) showed more inhibition than other extracts. Chloroform extract (O.D. 1.53)

showed more inhibition than Hexane extract (O.D. 1.59). In the case of *Salmonella typhimuruim*, all extract showed good inhibitory effect with respect to control (O.D. 1.70). Maximum inhibition was found in case of water extract (O.D. 1.49). Ethanol extract (O.D. 1.56) also showed good inhibitory effect but showed less inhibition than water extract. Chloroform extract (O.D. 1.67) and Hexane extract (O.D. 1.66) showed very less inhibition. In the case of *Staphylococcus epidermidis* in comparison to control (O.D. 1.70), water extract (O.D. 1.30) have showed more inhibition than other extracts. Ethanol extract (O.D. 1.56) also showed good inhibition. Chloroform extract (O.D. 1.67) was found to be least effective. The effect of different extracts on various gastrointestinal pathogens showed variable results that were in accordance with the earlier studies (Kannan, 1996; Farrukh and Ahmad, 2003; Sharma and Singh, 2002). Antibacterial properties of ethanolic and aqueous extracts might be due to the presence of alkaloids as alkaloids are natural antioxidants. All other extracts were observed to show good microbicidal properties. All observation and statistical data showed a clear-cut view about the activity of different extracts. This might be useful in formulating the antimicrobial potential of *Thuja occidentalis*.

Thuja occidentalis extract was also found to enhance humoral immune responses on 7th day by 18% as compared to the control, Levamisol, which showed 27%. The plant extract showed an enhancement in cell mediated immune responses on 7th day, by 15.37% as compared to control, Levamisol, which showed 23.65%. Total WBC count was also altered in Swiss mice treated with the *Thuja occidentalis* extract (Table 1). The effect of methanolic extract of the plant on the haematological parameters of the tumour bearing mice showed an increase in number of RBCs but a decrease in WBCs compared to the control mice. These data were based on the differential leucocyte count by Leishman staining. Methanolic plant extract treated organisms showed enhanced life span proving the improvement in immunomodulatory potentials (Table 2). Plants are

Table 1: Effect of methanolic extract of *Thuja occidentalis* on haematological parameters

Treatment	Total WBC count ($x10^3$)μl^{-1}	Total RBC count ($x10^5$) μl^{-1}
Cancer control	6.99±0.34	3.19±0.13
Extract treated	5.22±0.56	3.48±0.03
5-FU treated	6.11±0.78	2.97±0.97
Normal control	3.77±0.54	5.1±0.67

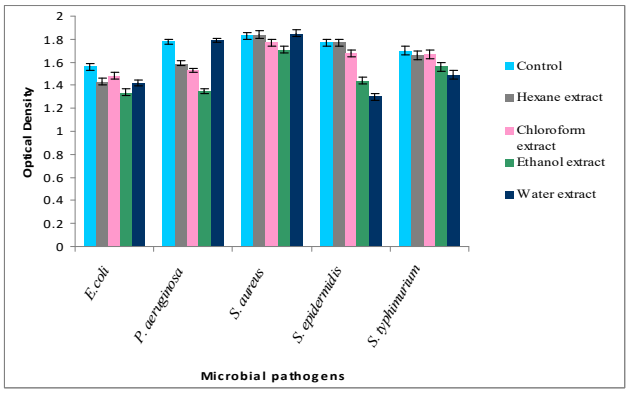

Fig. 1 Antimicrobial activity of different *Thuja occidentalis* extracts against gastrointestinal pathogens. The values represented the mean optical density ± SE

Table 2: Effect of methanolic extract of *Thuja occidentalis* on life span of tumour induced mice

Treatment	Number of animal with tumour	Number of days survived
Cancer control	6/6	17±0.4
Extract treated	6/6	23±0.9
5-FU treated	6/6	25±1.8

*** Values are represented as means ±SD**

the best friend of human being and are big source of natural medicines. *Thuja occidentalis* is one of those plants that are not only being used in traditional medicines but also as ornamental plant, from many years (Chang *et al*., 2000). The findings in the present study suggested that the plants possess high immunomodulatory and antimicrobial properties that are in accordance with the earlier studies. In folk medicine, *Thuja*

occidentalis has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism (Shimada, 1956; Baran, 1991; Offergeld *et al*., 1992). Today, it is mainly used in homeopathy as mother tincture or dilution. 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 and as an adjuvant to antibiotics in severe bacterial infections such as bronchitis, angina, pharyngitis and sinusitis (Von Blumroder, 1985). Thus the plant has a high potential that might be used in several medicinal formulations to make herbal medicines.

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