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Original Research Article

Evaluation of antimicrobial potential of successive extracts of *Ulmus wallichiana* Planch.

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ABSTRACT

Background: The plant *Ulmus wallichiana* Planch. is found in hills of Uttarakhand, India. Bark of *U. wallichiana* is commonly used as traditional healer for bone fracture of animals as well as human beings and also used as wound healer remedy.
Objective: The present study was designed to evaluate antimicrobial potential of various extracts of *U. wallichiana* bark.
Materials and methods: Soxhlet extraction method was used for preparation of different extracts viz. petroleum ether, chloroform, ethyl acetate, ethanol and aqueous. Antioxidant activity was determined by DPPH and FRAP assay method. *In vitro* antimicrobial activity was evaluated using agar well diffusion method.
Results: Ethyl acetate extract exhibited the highest significant antioxidant activity. Antibacterial activity was performed against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa.* Amongst the various extracts tested, only ethyl acetate exhibited highest zone of inhibition as compared to other extracts and greater than standard drug. Chloroform extract also showed moderate zone of inhibition. Antifungal activity was evaluated against *Aspergillus fumigates* and *Aspergillus flavus.* The ethyl acetate extract showed maximum zone of inhibition as compared to other extracts. Chloroform extract showed mild antifungal activity. Chloramphenicol and nystatin were used as a positive control as antibacterial and antifungal agent respectively. Furthermore, the highest percentage of phenolic and flavonoid compounds was estimated in ethyl acetate extract.
Conclusion: The ethyl acetate extract of *U. wallichiana* showed the highest antimicrobial activity, and should be further investigated for isolating active compound(s) responsible for antimicrobial activity.

1. Introduction

In the current scenario, there have been increasing antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant [1]. The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality [2]. Therefore, there is an urgent need to look out for new substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents of plant origin, with the aim of discovering potentially useful active phytoconstituents that can serve as a source and template for the synthesis of new antimicrobial agents [3].

The plant *Ulmus wallichiana* Planch. (family: Ulmaceae) found in western Himalayan regions of India, is one of the richest emporiums for medical taxa. Traditionally, *U. wallichiana* has been used in the treatment of digestive tract diseases [4]. Bark of this tree is commonly used for bone fracture healing of animals as well as human beings as folk medicine in Uttarakhand (India) [5]. Bark paste of the plant is mentioned in drugs with the potential of wound healing [6]. Other uses of the plant in public domain include treatment of health related disorders with osteoporosis. Leaves are used as fodder for sheep and goats in Jammu and Kashmir (India) [7].

Phytochemically, the plant *U. wallichiana* contains aliphatic hydrocarbon and triterpenes (Masoodi et al., 2013). Some other allelopathic compounds identified are alnulin, betulin, caffic acid,

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catechol, lupenol, ferulic acid, scopoletin and vanillin [8]. A flavonol 2S, 3S-Aromadendrin-6-C-β-D-glucopyranoside [9], ulmoside A, B and Naringenin-6-C-beta-glucopyranoside are also reported in the bark [10]. Despite a long history of use of *U. wallichiana* as a traditional medicine for the treatment of various ailments, especially for it's wound healing property, the plant has never been subjected to antimicrobial activity studies. Thus, it was considered worthwhile to evaluate *U. wallichiana* for antimicrobial potential.

2. Material and methods

2.1. Plant material

The plant material (*U. wallichiana*) was collected from near, Budhar, Budhakedar Nath Ghansali, Tehri Garhwal, Uttarakhand (India). The plant was identified and authenticated at the herbarium of Botanical Survey of India, Dehradun (India) vide reference no. BSI/NRC Tec/Herb (Ident.)/2016-17/455.

2.2. Preparation of extracts

The dried parts of the plant *U. wallichiana* (barks) were collected, and then pulverized through a mechanical grinder. The powdered material was dried in hot air oven at moderate temperature. The powdered material was subjected to successive Soxhlet extraction by solvent in increasing polarity viz. petroleum ether, chloroform, ethyl acetate, methanol and then macerated with water. After that, extract was concentrated and stored at 4 °C until further use in the equipment.

2.3. Determination of total phenolic and flavonoid content

2.3.1. Determination of total phenolic content

One mg/ml of plant extracts were prepared in methanol and then, diluted with 10 ml of distilled water. Then, 1.5 ml of Folin Ciocalteu reagent was added and allowed to incubate at room temperature for 5 min. 4 ml of 20% w/w sodium carbonate solution was added in each test tube and then, further adjusted with distilled water up to the mark of 25 ml, agitated and left to stand for 30 min at room temperature. Absorbance of test samples was measured at 765 nm using spectrophotometer against blank (distilled water) [11].

The phenolic content was calculated with the help of standard curve equation and the formula is given below (Ayurvedic pharmacopeia of India, 2008).

$$\text{Total phenolic content}(\%\ \text{w/w}) = \frac{\text{GAE} \times \text{V} \times \text{D} \times 10^{-6}}{\text{w}} \times 100$$

where

GAE – Gallic acid equivalent (micro gram/ml)
V – Total volume of sample (ml)
D – Dilution factor
W – Sample weight (gm)

2.3.2. Determination of total flavonoid content

The total flavonoid content of all extracts of *U. wallichiana* was estimated according to the aluminum chloride method as follows: Aliquots of extracts 1 mg/ml solution was taken and the diluted standard solution (0.5 ml) was separately mixed with 1.5 ml of methanol (95%), 0.1 ml of aluminum chloride (10%), 0.1 ml of 1 M (potassium acetate) and 2.8 ml of distilled water. Absorbance at 415 nm was recorded after 30 min of incubation against blank (distilled water). The concentration of flavonoid in the test samples was calculated and expressed as mg quercetin equivalent/g of sample [12].

The total flavonoid content was expressed in milligrams of rutin equivalent per gram of extracts.

Percentage of total flavonoid content calculated in mg/g as:

$$\text{Total flavonoid content}(\%\text{w/w}) = \frac{\text{RE} \times \text{V} \times \text{D} \times 10^{-6}}{\text{w}} \times 100$$

where

RE – Rutin equivalent (micro gram/ml)
V – Total volume of sample (ml)
D – Dilution factor
W – Sample weight (gm)

2.4. Antioxidant activity

2.4.1. DPPH assay

Free radical scavenging activity was measured by the spectrophotometric method. Stock solution of DPPH (2, 2 di-phenyl 1-picryl hydrazyl) (15 mg in 10 ml methanol) was prepared such that 75 μl of it in 3 ml of methanol gave an initial absorbance of 0.9. Decrease in the absorbance in presence of sample extract at different concentration (0.2, 0.4, 0.6, 0.8 and 1 ml) was noted after 15 min. IC_{50} was calculated from % inhibition [13].

2.4.1.1. Protocol for DPPH free radical scavenging activity

1. Preparation of stock solution of test sample: 100 mg test sample was dissolved in 100 ml methanol to get 1000 μg/ml solution.
2. Dilution of test solution: 1, 2, 3, 4 and 5 stock were taken and diluted up to 10 ml methanol to get 200, 400, 600, 800 and 1000 μg/ml solution.
3. Preparation of DPPH solution: 15 mg of DPPH was dissolved in 10 ml of methanol. The resulting solution was covered with aluminum foil to protect from light.
4. Estimation of DPPH scavenging activity:
   a) 75 μl of DPPH solution was taken and the final volume was adjusted to 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading.
   b) 75 μl of DPPH solution and 50 μl of the test sample of different concentrations were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol.
   c) Absorbance at zero time was taken in UV–visible at 517 nm for each concentration.
   d) Final decrease in absorbance of DPPH with sample of different concentrations was measured after 30 min at 517 nm.
   e) 75 μl of DPPH and 50 μl of methanol were used as a negative control and single methanol blank.
   f) Percentage inhibitions of DPPH radical by test compound were determined by the following formula:

$$\%\ \text{Reduction} = \frac{\text{Control absorbance} - \text{Test substance}}{\text{Control substance}} \times 100$$

   g) The determined percentage inhibitions were used against the concentration of the sample to build a regression fit.

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