

Antibacterial Evaluation and Phytochemical Analysis of *Betula utilis* D. Don Against Some Human Pathogenic Bacteria

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Abstract: Successive solvent extract viz., petroleum ether, chloroform, methanol, ethanol and water extracts of bark of *Betula utilis* D. Don was evaluated for antibacterial activity, against fourteen important bacterial strains by agar-well diffusion method. Methanol extract was showed significant activity against all the tested bacteria followed by ethanol and aqueous extract. Chloroform and Petroleum ether extracts did not show any activity. The antibacterial activity is more significant in solvent extracts compared to aqueous extract in all the plants indicating that the active principle responsible for antibacterial activity is more soluble in organic solvents. Comparison of the inhibitory activity of the extracts with the antibiotics gentamicin revealed that methanol extracts of *Betula utilis* was significantly higher than that of the antibiotics tested. The results suggest that *Betula utilis* is scientifically validate the use of this plant in the traditional medicine for isolation and characterization of the active principle for further exploitation in medical microbiology.

Key words: *Betula utilis* D. Don • Antibacterial activity • Methanol extract • Phytochemical analysis

INTRODUCTION

Herbal medicine represents one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way [1].

Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases. For example, vincristine (an antitumor drug), digitalis (a heart regulator) and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials [2]. Thousands of secondary plant products have been identified and it is estimated that thousands of these compounds still exist. Since secondary metabolites from natural resources have been elaborated within living systems, they are often perceived

as showing more “drug – likeness and biological friendliness than totally synthetic molecules” making them good candidates for further drug development [3-5]. Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens.

Betula utilis D. Don (Betulaceae) is a moderate-sized tree that grows up to 20m in height. The bark is smooth, shining, reddish white or white, with white horizontal lenticels. The outer bark consists of numerous thin papery layers, exfoliating in broad horizontal rolls. The inner cortex is red and moist. The leaves are ovate-acuminate, elliptic and irregularly serrate. The flowers bloom in May-June, in pendulous spikes. Seeds are thin and winged. Its therapeutic Antiseptic, aromatic, carminative and contraceptive. The bark contains betulin, lupeol, oleanolic acid, acetyloleanolic acid, betulic acid, lupenone, sitosterol, methyle betulonate, methyl betulate and a new triterpenoid, karachic acid. It is aromatic and has antiseptic properties [6, 7].

MATERIALS AND METHODS

Plant Collection and Extraction: *Betula utilis* D. Don bark was collected from Amruthkesari Depot, Bangalore (India). Barks was washed thoroughly 2-3 times with

running tap water and once with sterile water, shade dried, powdered and used for extraction. Plants identified and authenticated by Prof. Balakrishnagouda, GKVK, Bangalore (India). The dried powder material was extracted successively with petroleum ether, chloroform, methanol, ethanol and water in the increasing order of their polarity [8]. The solvent was removed under pressure to obtain a total extracts. Yields were 4.4, 9.4, 7.0, 7.0 and 13% in petroleum ether, chloroform, methanol, ethanol and water respectively. All the extracts were subjected to antibacterial activity assay.

Human Pathogenic Bacterial Cultures: *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109), *Proteus mirabilis* (MTCC 1429), *Pseudomonas aeruginosa* (MTCC 1688), *Salmonella paratyphi A* (MTCC 735), *Salmonella typhi* (MTCC 733), *Salmonella typhimurium* (MTCC 98), *Shigella flexneri* (MTCC 1457), *Shigella sonnei* (MTCC 2957), *Staphylococcus aureus* (MTCC 737), *Streptococcus faecalis* (MTCC 459) and authentically identified clinical isolates of *Citrobacter* sp., *Salmonella paratyphi B* and *Shigella boydii* were obtained from the Department of Microbiology, Government Medical College, Mysore, India. All test strains were maintained on nutrient agar slopes (Hi-Media Laboratories Pvt. Limited, Mumbai, India) at room temperature and were sub-cultured, every two-weeks. These bacteria served as test pathogens for the assay.

Antibacterial Activity Assay: Antibacterial activity of aqueous extracts and solvent extracts was determined by cup diffusion method on nutrient agar medium [9]. Cups were made in nutrient agar plate using sterile cork borer (5 mm) and inoculum containing 10^6 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50 μ l each of all aqueous and solvent extracts were placed in the cups made in inoculated plates. The treatments also included 50 μ l of sterilized distilled water and methanol separately which served as control. Antibiotics Gentamicin 10mcg/disc (5 mm in diameter) of Hi-Media Laboratories were also treated for activity for comparative efficacy. The plates were incubated for 24 hours at 37°C and zone of inhibition if any around the wells was measured in mm (millimeter). For each treatment six replicates were maintained. The means were analysed by one way analysis of variance (ANOVA) followed by Turkey's multiple comparison test using Graphpad prism 4.

Phytochemical Analysis: Phytochemical analysis of petroleum ether, chloroform, methanol, ethanol and water

extract for presence/absence of metabolites such as carbohydrates, alkaloids, glycosides, flavonoids, tannins, steroids, saponins, triterpenoids, protein, resins, fixed oils and fats was carried out [10].

RESULTS

Antibacterial Activity: Different solvents and aqueous extracts tested at 50 μ l concentrations against fourteen important human pathogenic bacteria are presented in Table 1. Among four solvent extracts (viz., petroleum ether, chloroform, methanol and ethanol) the tested against fourteen human pathogenic bacteria methanol and ethanol extracts recorded significant antibacterial activity against all the test pathogens. Antibacterial activity was not observed in petroleum ether and chloroform extracts against all the pathogens.

Among methanol and ethanol extracts, methanol extracts recorded significant antibacterial activity followed ethanol. *Shigella boydii* found highly susceptible to methanol extract, where as *Proteus mirabilis* was less susceptible to both methanol and ethanol extracts *Pseudomonas aeruginosa* was found highly susceptible to ethanol extract. Methanol extract exhibited similar antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella paratyphi A* and *Shigella sonnei* where it was around 16 mm and 14 mm inhibition zone was observed against *Citrobacter* sp., *Salmonella paratyphi B*, *Salmonella typhimurium*, *Salmonella paratyphi*, *Staphylococcus aureus* and *Streptococcus faecalis*. Zone of inhibition for ethanol extract against test pathogens varied significantly.

Antibacterial activity of aqueous extract varied greatly among the different test pathogenic bacteria. Highest antibacterial activity was observed against *Shigella sonnei* followed by *Salmonella typhi*, even though antibacterial activity was observed against other pathogenic bacteria also it was not found significant. Inhibition zone more than 10 mm were against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Streptococcus faecalis*.

Aqueous extract recorded significant antibacterial activity against *Pseudomonas aeruginosa* compared to gentamicin, where as against other test pathogens it was not significant. Among different solvent extracts both methanol and ethanol extract recorded highly significant antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* compared to gentamicin. Antibacterial activity of methanol extract against *Citrobacter* sp. is almost equal compared to gentamicin.

Table 1: Antibacterial activity of different extracts of *Betula utilis* D. Don on human pathogenic bacteria

Human pathogenic bacteria	Zone of inhibition in mm(Mean±SD)					
	PE	C	M	E	W	GEN
<i>Citrobacter</i> sp.	-	-	14.00±0.18 ^a	13.50±0.19 ^a	08.50±0.18 ^b	14.3±0.50 ^a
<i>Escherichia coli</i> MTCC 443	-	-	16.25±0.25 ^a	15.13±0.00 ^b	07.60±0.00 ^c	13.3±0.40 ^{db}
<i>Klebsiella pneumoniae</i> MTCC109	-	-	13.70±0.19 ^a	13.20±0.19 ^a	11.70±0.19 ^b	14.5±0.40 ^a
<i>Pseudomonas aeruginosa</i> MTCC1688	-	-	16.50±0.19 ^a	15.50±0.19 ^a	10.50±0.18 ^{cd}	9.5±0.30 ^d
<i>Proteus mirabilis</i> MTCC1429	-	-	10.25±0.19 ^a	09.50±0.00 ^a	07.50±0.00 ^b	13.6±0.30 ^c
<i>Salmonella typhi</i> MTCC733	-	-	13.50±0.19 ^a	12.70±0.00 ^a	12.70±0.00 ^a	19.1±0.20 ^b
<i>Salmonella paratyphi</i> A MTCC735	-	-	14.63±0.18 ^a	12.00±0.19 ^b	06.00±0.19 ^c	17.5±0.20 ^d
<i>Salmonella paratyphi</i> B	-	-	16.00±0.27 ^a	15.25±0.00 ^a	08.27±0.00 ^b	20.5±0.30 ^c
<i>Salmonella typhimurium</i> MTCC98	-	-	14.20±1.38 ^a	13.50±0.00 ^a	11.50±0.00 ^b	15.5±0.30 ^{ad}
<i>Shigella boydii</i>	-	-	17.50±0.19 ^a	14.75±0.25 ^b	09.25±0.25 ^c	20.5±0.60 ^d
<i>Shigella flexneri</i> MTCC1457	-	-	11.50±0.19 ^a	10.90±0.00 ^a	08.50±0.00 ^b	13.5±0.40 ^c
<i>Shigella sonnei</i> MTCC 2957	-	-	16.60±0.18 ^a	15.20±0.00 ^a	15.20±0.00 ^{ac}	17.5±0.50 ^{ab}
<i>Staphylococcus aureus</i> MTCC737	-	-	14.13±0.23 ^a	12.13±0.44 ^a	08.25±0.44 ^b	21.6±0.20 ^c
<i>Streptococcus faecalis</i> MTCC459	-	-	14.00±0.46 ^a	12.25±0.00 ^b	11.25±0.00 ^c	17.5±0.30 ^d

PE-Petroleum ether, C-Chloroform, M-Methanol, W-Water extract, GEN-Gentamicin.

Gentamicin disc (10µg) as a positive reference standard; Values are mean inhibition zone (mm) ± S.D of six replicates. Means within a row followed by same letters are statistically non-significant by tukey's test at p = 0.05; - no activity. 50 µl was applied to each well. Concentration of the extracts was 100mg/ml

Table 2: Preliminary phytochemical analysis of *Betula utilis* D. Don bark extracted with different solvents

Phytoconstituents	PE	C	M	E	W
Carbohydrates	+	+	+	+	+
Alkaloids	+	+	+	+	+
Glycosides	-	-	+	-	-
Flavonoids	-	-	-	-	-
Tannins	-	-	-	-	-
Steroids	+	-	-	-	-
Saponins	-	-	-	-	-
Triterpenoids	-	-	-	-	-
Protein	-	-	-	-	-
Resins	-	-	-	-	-
Fixed oils and fats	-	-	-	-	-

+ Present, -Absent, PE-Petroleum ether, C-Chloroform, M-Methanol, W-Water extract

Phytochemical Evaluation: Phytochemical analysis of all the extracts revealed that carbohydrates and alkaloids are generally present in all the extracts. Glycosides were found only in methanol extract and steroids in petroleum ether extract. Other metabolites such as flavonoids, tannins, saponins, triterpenoids, protein, resins, fixed oils and fats were absent in all the extracts (Table 2).

DISCUSSION

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. In recent years development of multidrug resistance in the pathogenic bacteria and parasites has created major clinical problems in the treatment of infectious diseases [11]. This and other problems such as toxicity of certain antimicrobial drugs on the host tissue [12, 13] triggered interest in search of new antimicrobial substances/drugs

of plant origin. Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant extracts for their anti-microbial activity may provide new anti-microbial substances; hence the present investigation clearly reveals the antibacterial nature of this plant and suggests that this plant could be exploited in the management of diseases caused by these bacteria in human systems.

It is interesting to note that antibacterial activity was highly pronounced in solvent extract compared to aqueous extract. It is also important to note that susceptibility of the pathogens was varied to solvent extract and aqueous extract. This indicates the presence of more than one active principle in *Betula utilis*. Plants are rich reservoir of antimicrobials [14] it is observed that a single plant is known to contain several active principles of biological significance [15]. The present finding is hence highly encouraging in recognizing a plant of interesting antibacterial activity.

Comparative efficacy with gentamicin is also highly encouraging. Two important pathogens viz., *E. coli* frequently associated with urinary track infection a common problem in stressed human being and office bearers who share common toilets[16] and *Ps. aeruginosa* frequently associated with infant bacteria is highly susceptible to methanol[17] and ethanol extract of *B. utilis* compared to gentamicin since the plant is of folkloric origin and is already in use as a medicinal plant with minimum and least toxicological studies the plant could be exploited against these pathogens of highest clinical importance. The present study records the scientific validation of this plant for use as an anti-infective agent. The present study also gives for the first time the

phytochemical profile of all the extracts used in the present study.

Literature survey reveals that phytochemical analysis of *Betula utilis* D. Don is lacking hence the present study is the first to describe its phytochemical analysis which revealed the presence of carbohydrates and alkaloids in all the extracts where as antibacterial activity was observed only in methanol and ethanol extracts. Since alkaloids are the secondary metabolites, whose function is known to be defensive to the plant. Further work needs to be carried out to confirm where the alkaloids are responsible for the desired activity. This also serves as important information for further isolation and purification of the active principles/principle from this plant.

The present study is successful in identifying candidate folkloric plant with heat stable constituents, since, all the extracts were subjected to heat treatment and then subject to antibacterial activity. Constituents were also found to be bactericidal since no growth was observed even after 48 hour of incubation in zone of inhibition area. In addition, this result form a good basis for selection of the plant for further phytochemical and pharmacological investigation and suggests antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their antimicrobials constituents and carry out further pharmacological evaluation.

Since compounds of biological origin are known to possess minimal residual effect. The most active extracts can be subjected to isolation of the therapeutic and carry out further pharmacological evaluation.

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