

INHIBITORY ACTIVITY OF METHANOLIC EXTRACT OF *ARAUCARIA HETEROPHYLLA* LEAVES AGAINST GRAM NEGATIVE BACTERIA

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Abstract

This study investigated antibacterial activity of methanolic extract of *Araucaria heterophylla* leaves against two gram-negative bacteria *Salmonella typhi* and *Escherichia coli* by disk diffusion method. At the initial investigation, the test sample extract did not show inhibitory activity against *Staphylococcus aureus* gram positive bacteria. So, in the next step observation was made against above mentioned gram-negative bacteria. Test sample was taken at a concentration of 500 µg/disk. A broad spectrum antibiotic Kanamycin (30 µg/disk) was used as standard to get comparable antibacterial activity. Weak antibacterial activity was shown in the test sample against *S. typhi* and *E. coli* with zone of inhibition 8 mm and 10 mm respectively. Although Kanamycin showed antibacterial activity against *S. typhi* and *E. coli* with zone of inhibition 32 mm and 35 mm correspondingly.

Key words: *Araucaria heterophylla*, Extraction, Antibacterial, *Escherichia coli*, *Salmonella typhi*, Antibiotic resistance, Zone of inhibition.

A great percentage of world's population partially or entirely relies on botanicals to treat human diseases and infections (Sofowora, 1993). The plants whether the parts or in whole are used to treat and manage different types of diseases from the ancient times. Folk practitioners have been using plant extracts for the treatment of various diseases since antiquity (Chitravadivuet *et al.*, 2009). Modern pharmacopoeias contain approximately 25% of drugs which are derived from plant sources (Adhikari and Paul, 2018). Several popular drugs of the past 50 years have been isolated from plants (Daret *et al.*, 2017). Medicinal compounds derived from plant created much attention against antibiotic resistance as they are well investigated for their efficacy and are being used to treat different microbial diseases (Sultana *et al.*, 2016). Bangladesh is a country that has different types of plants. It is estimated that almost 1000 plants of Bangladesh have medicinal values (Alamgir and Fatema, 2013). *Araucaria heterophylla* is most commonly known as Norfolk Island pine or Christmas tree plant which belongs to Araucariaceae

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family (Patil *et al.*, 2013). Out of 19 species of the genus *Araucaria*, some are used as decorative plants and for timber uses while some species have medicinal values respectively. It is available almost everywhere in Bangladesh. It is mostly distributed throughout Belize, China, Ecuador, El Salvador, Honduras, Venezuela (Aslam *et al.*, 2013). The study showed that resin obtained from *A. heterophylla* plant possesses moderate activity against colon and breast cancer cell lines (Sattar *et al.*, 2009). *A. heterophylla* plant is usually not attacked by insects and microorganisms. The study showed that alcoholic extract of *A. heterophylla* has antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris* (Goud *et al.*, 2017). *E. coli* are gram-negative bacteria of Enterobacteriaceae family. Most strains of *E. coli* are harmless but some are pathogenic which are responsible for diarrheal infections, neonatal meningitis, septicemia, and urinary tract infections (UTIs).

The route of infection is fecal-oral through contaminated food and water (Makvana and Krilov, 2015). A study showed a significant increase in ciprofloxacin resistance of *E. coli* (Pormohammad, A. *et al.*, 2019). Approximately over 26 million people are culture positive for *S. Typhi*/ *Paratyphi* every year and a big percentage of isolates are resistant to numerous antimicrobials. Enteric fever continues to be critical hubs in South and South-East Asia, dominated by the H58 haplotype of *S.typhi* in many areas. Fluoroquinolone resistance is widely increased across Asia in particular, because of the extensive use of this category of antimicrobials (Britto *et al.*, 2018).

A. heterophylla powder obtained from the leaves of this plant belonging to the family Araucariaceae has been taken up to explore our study.

Leaves of *A. heterophylla* were collected from Ranada Prasad Shaha University campus, Shitalakhya, Narayanganj, in August, 2019 and was identified by Bangladesh National Herbarium, Mirpur, Dhaka. DACB Accession no is 48435.

After collection, the leaves were cleaned gently. Then all the leaves were allowed for shade drying in clean area. After that leaves were dried by oven for 24 hours at 40°C to facilitate grinding process. When the leaves were dried properly it was ground to very small pieces by using a domestic grinder. Then leaf powder was ready for extraction.

Approximately 300g of powder materials were soaked in sufficient amount of methanol for about two weeks. The containers with its contents was sealed and kept for 7 days with frequent stirring. After vacuum filtration, the filtrate was evaporated to get dark green, crude methanolic extract. Approximately 15.6% extract came out from 300g plant. Crude extract was stored for further biological investigations.

By using disk diffusion method, antibacterial activity of plant extract was carried out. (Bauer *et al.*, 1966). Each chemicals and reagents used in the study were of analytical grade. Selected gram-negative bacteria (*Salmonella typhi*, *Escherichia coli*) were sourced from BCSIR (Bangladesh Council of Scientific and Industrial Research). A few colonies (3 to 10) of the organism to be tested were collected with a wire loop from the original

culture plate and introduced into a test tube containing 4 ml of tryptose phosphate or trypticase soy broth. To form a bacterial suspension of moderate cloudiness, these tubes were incubated for about a time span of 2 to 5 hours. The suspension was then diluted with distilled water to a density visually identical to that of a standard, prepared by adding 0.5 ml. of 1 per cent BaCl₂ to 99.5 ml. of 1 per cent H₂SO₄ (0.36 N). For sensitivity plates large 15 cm Petri dishes were used with Mueller-Hinton agar (5 to 6 mm in depth). Before inoculation, plates were dried for about 30 minutes and were used within 4 days of preparation. With the help of a cotton swab, the bacterial broth suspension was spread evenly in 3 planes onto the surface of the medium. Excessive suspension was extracted from the swab by being rotated against the side of the tube before the plates were seeded. Standard Kanamycin (30 µg/disk), blank disk containing only solvent and test sample disk (500 µg/ disk) were prepared. After the inoculum had dried (3 to 5 minute), the disks were placed on the agar with flamed forceps and gently pressed down to ensure contact. Within 30 minutes, plates were incubated. After overnight incubation, the zone diameters (including the 6-mm disk) were measured with calipers near the agar surface. A reading of 6 mm indicates no zone (Bauer et al., 1966).

Methanolic extract of *A. heterophylla* leaves (MAH) showed weak antibacterial activity against *S. typhi* and *E. coli* with zone of inhibition 8 mm and 10 mm respectively at 500µg/disk concentration compared to standard Kanamycin (30µg/disk) with zone of inhibition 32 mm and 35 mm respectively. The diameters of zone of inhibition against the tested microorganisms is shown in table 1.

Table 1: Antibacterial activity of MAH

Name of Test Sample/Standard	<i>Salmonella typhi</i> (Zone of Inhibition in mm)	<i>Escherichia coli</i> (Zone of Inhibition in mm)
MAH	8	10
Kanamycin	32	35

The results apparently showed the potentiality of the extract to inhibit both gram negative bacterial strain. So, the crude extract has weak antibacterial activity in terms of gram-negative bacteria.

Based on test results, it can be said that *A. heterophylla* plant is not only a decorative plant but also possesses medicinal value. This study opens the gate to investigate both phytochemically and pharmacologically thereby exploring medicinal potentials of *A. heterophylla* plant.

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