

Original Research Article

Antibacterial activity of organic and aqueous extracts of *Hopea odorata* leaves and their total flavonoid content.

Abstract

Objective: To examine the therapeutic effects of different extracts of *Hopea odorata* leaves against bacteria and to determine their total flavonoid content.

Methods: Leaves of *Hopea odorata* was extracted with pure methanol (MEHO), ethanol (EEHO) and water (AEHO), which are tested for antibacterial activity on three gram positive and three gram negative bacteria by disk diffusion method. Total flavonoid content determined based on the method of Wang et al.

Result: Among the all extracts, MEHO exhibited strong antibacterial activity. Where it showed zone of inhibition in highest concentration of 2 mg/ml against Gram-positive bacteria *Staphylococcus aureus* (20mm), *Bacillus subtilis* (18mm), *Bacillus Megaterium* (15mm) and Gram-negative bacteria *Salmonella typhi* (18mm), *Escherichia coli* (14mm), *Shigella dysenteriae* (19mm). The content of flavonoid good at all extracts, but MEHO (85.98±0.76 mg quercetin/g) contained highest among them. For both of experiment, activity found as follows, MEHO > EEHO > AEHO.

Conclusion: These findings suggest that the plant may be a possible source for the development of new antibacterial agent and flavonoid may one of such phytochemical, which exhibit antibacterial activity.

Keywords: *Hopea odorata*, antibacterial, disc diffusion method, flavonoid, gram negative

1 Introduction

Plants are rich source of antimicrobial and antioxidant agents. The studies of medicinal plants fascinated many researchers, and the plants have great prospective in treating human diseases such as cancer, coronary heart diseases, diabetes and infectious diseases. About 25% of prescribed drugs in the world originate from plants ^[1] and over 3000 species of plants have been reported to have both antibacterial ^[2]. Apart from being an antibacterial few medicinal plants also exhibit antiparasitic activity ^[3]. Antibacterial resistance has become a worldwide problem. Approximately 20% of the plants have been submitted to pharmacological tests, and a number of new antibiotics introduced to the market are obtained from natural or semi-synthetic resources. According to World Health Organization (WHO), more than 80% of the world's population relies on medicinal plants for their primary health care needs and also for the treatment of infectious diseases ^[4].

Flavonoids are a major class of oxygen-containing heterocyclic natural products that are widespread in green plants.^[5] Generally, they are found as plant pigments in a broad range of fruits and vegetables.^[6]

These are C₁₅ compounds composed of two aromatic rings linked through a three-carbon bridge with a carbonyl functional group located at one end of the bridge. Flavonoids have been recognized as having a protective effect in plants against microbial invasion by plant pathogens.^[7,8] Flavonoid-rich plant extracts have been used for centuries to treat human disease.^[9] Isolated flavonoids have been shown to possess a host of important biological activities, including antifungal and antibacterial activities.^[10-12] The potential of naturally occurring flavonoids as anti-infective agents has been recognized.^[13] However, reports of activity in the field of antibacterial flavonoid research are widely conflicting, probably owing to inter- and intra-assay variations in the susceptibility testing.^[9]

Hopea Odorata belongs to the Dipterocarpaceae family, locally known as Telsur (Bangladesh). It can grow up to 120 feet to produce good quality timber. The wood of *H. Odorata* varies in color from a very pale yellow, or white to brown when first cut and characteristically darkens to a brownish or yellowish-brown color after more or less prolonged exposure to the air. The dammar of this tree is said to have medicinal property used in treating sores and wounds.^[14] Photochemistry studies reported that the heartwood of *H. Odorata* contain certain types of phenolic compounds.^[15] These polyphenols are reported useful as antioxidants, antimutagens, scavengers of free radicals and therefore have implications in the prevention of pathologies such as cancer and cardiovascular disease.^[16] The aim of the present study to identify the antibacterial activity of methanol, ethanol and aqueous extract of *Hopea odorata* leaves and determine total flavonoid content.

2 MATERIAL AND METHODS

2.1 Plant material:

Fresh leaves of *Hopea odorata* were collected from area of University of Chittagong, Chittagong, Bangladesh in the month of November 2014. It was authenticated by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

2.2 Preparation of Extract:

The leaves were dried for a period of 10 days under shade and ground. The ground leaves (400 gm) were soaked in sufficient amount of methanol, ethanol and distilled water separately for one week at room temperature with occasional shaking and stirring then the whole mixture was filtered and the filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterlin Ltd, UK) to get a viscous mass. The viscous mass was kept at room temperature under a ceiling fan to get a dried extract (about 8%). The extract prepared was for pharmacological screening.

2.3 Chemicals and reagents

All other chemicals and reagents were of analytical grade. Ethanol and methanol were purchased from Merck (Germany). Aluminium chloride and potassium acetate were purchased from Fluka (Fluka chemie

GmbH, CH-9471 Buchs). Quercetin was purchased from BDH Chemicals (BDH Chemicals Ltd. Poole, England). Kanamycin (30µg/disc, Oxoid, England) was used as a standard antibiotic disc.

2.4 *In vitro* Antibacterial activity

2.4.1 Bacterial strains

Gram positive *Bacillus subtilis* (*B. subtilis*), *Bacillus Megaterium* (*B. megaterium*), *Staphylococcus aureus* (*S. aureus*) and Gram negative *Escherichia coli* (*E. coli*), *Salmonella typhi* (*S. typhi*) and *Shigella dysenteriae* (*S. dysenteriae*) were used for antimicrobial screening. All the stock cultures were collected from Department of Microbiology, Pharmic Ltd, Chittagong, Bangladesh.

2.4.2 Media preparation and maintenance of bacteria

All of the bacterial strains were grown and maintained on Nutrient agar (Merck, India) media at 37 °C and pH (7.4±0.2). The bacteria were subcultured overnight.

2.4.3 Preparation of concentration

In the study of the antibacterial activity, all the extracts were diluted in their solvent. So methanol extract diluted in methanol and other also. The concentrations corresponding to the extracts given in Table1 are expressed in terms of µg/ disk.

2.4.4 Preparation of discs

The discs of about 5 mm in diameter were cut by punching machine from Whatman No.1 filter paper. The discs were taken in a petri dish and sterilized by autoclaving, dried in oven at 180°C.

2.4.5 Antimicrobial screening by disk diffusion technique

The antibacterial effects were tested by the disc diffusion method ^[17] with some minor modification. The filter paper discs (about 5 mm in diameter) were individually impregnated with 50 µl of 0.5mg, 1mg, 1.5mg and 2 mg/disk of different extracts of the *H. odorata* leaves and then placed onto the agar plates which had previously been inoculated with the test microorganisms (within 15 min). The Petri dishes were kept at 4 °C for 3 h before incubation at 37 °C for 24 h. The diameters of the inhibition zones were measured in millimeters. All the tests were performed in duplicate. Blank disc impregnated with methanol, ethanol and distilled water was used as negative control and disc of Kanamycin (30µg/disc) as positive control.

2.5 Determination of total flavonoid content

Total flavonoid content of different extracts was evaluated with method of Wang^[18]. 1ml of *H. odorata* different extracts or standard of different concentrations was taken in a test tube and 3 ml of methanol was added. Then 200µl of 10% aluminium chloride solution was added into the same test tube followed by the addition of 200 µl of 1M potassium acetate. Finally, 5.6 ml of distilled water was mixed with the reaction mixture. The reaction mixture was then incubated for 30 minutes at room temperature to complete the reaction. Then the absorbance of the solution was measured at 415 nm using a spectra photometer against blank. Methanol served as blank. The Total content of flavonoid compounds in different extracts of *H. odorata* was expressed in mg/g quercetin equivalent (QE).

2.6 Statistical analysis

The results were expressed as mean from duplicate experiment for zone of inhibition and results were expressed as mean $\pm$ standard error for mean (SEM) from triplicate experiments for total flavonoid content and evaluated with the analysis of student's t-test using SPSS. Differences were considered significant at a level of $P < 0.05$ and $P < 0.001$.

3 Results

3.1 *In vitro* Antibacterial activity

The antibacterial activity of MEHO, EEHO and AEHO were tested against 6 pathogenic bacteria and exhibited a significant antibacterial activity against both gram positive and gram negative bacteria at the concentration of 0.5, 1.00, 1.5, and 2 mg/disc which is shown in **Table 1**. The inhibitory activities showed the test samples were compared with standard broad spectrum antibiotic Kanamycin (30µg/disc). The zone of inhibition produced by MEHO against gram-positive bacteria were found to be 7 mm to 20 mm and against gram-negative bacteria were found to be 8 mm to 19 mm at different concentration. EEHO produced zone of inhibition against gram-positive bacteria in the range of 7 mm to 18 mm and against gram-negative bacteria in range of 9 mm to 17 mm at different concentration. AEHO produced zone of inhibition against gram-positive bacteria in the range of 7 mm to 16 mm and against gram-negative bacteria in range of 7 mm to 15 mm at different concentration. On the other hand, Kanamycin showed a zone of inhibition against gram-positive bacteria in the range of 27 mm to 30 mm and against gram-negative bacteria in the range of 28 mm to 33 mm.

Table 1: Results of antibacterial activity testing of MEHO, EEHO and AEHO.

Test microorganism	Zone of inhibition (mm)			
	MEHO	EEHO	AEHO	Kanamycin

	0.5mg/disk	1mg/disk	1.5 mg/disk	2mg/disk	0.5mg/disk	1mg/disk	1.5 mg/disk	2mg/disk	0.5mg/disk	1mg/disk	1.5 mg/disk	2mg/disk	30 µg/disk
Gram Positive													
<i>Staphylococcus aureus</i>	14	16	17	20	10	13	15	18	9	12	15	16	30
<i>Bacillus subtilis</i>	8	12	15	18	8	11	13	15	7	10	12	14	27
<i>Bacillus Megaterium</i>	9	12	14	15	7	10	13	16	8	10	12	14	28
Gram Negative													
<i>Salmonella typhi</i>	9	12	14	18	10	13	15	17	7	10	13	15	33
<i>Escherichia coli</i>	8	8	10	14	9	10	12	15	7	8	10	12	28
<i>Shigella dysenteriae</i>	10	12	15	19	9	12	14	17	9	11	13	15	31

Bold text indicates the highest antibacterial activity of extracts on each test bacteria.

3.2 Total flavonoid content

The total flavonoid contents of the extracts are shown in **Table 2**. The total flavonoid content of *Hopea odorata* leaves was higher in plants at methanol extract, which was 85.98±0.76 mg quercetin/g. Other extracts of *Hopea odorata* also contain good amounts of flavonoid, EEHO (70.88±0.33mg quercetin/g) and AEHO (35.60±0.28 mg quercetin/g). So flavonoid content of different extracts of *Hopea odorata* leaves are as follows,
MEHO > EEHO > AEHO

Table 2: Contents of flavonoid (expressed as mg quercetin/g dry weight) in different extracts of *Hopea odorata* leaves.

Extract	Total Flavonoid(mg quercetin/g)
MEHO	85.98±0.76*
EEHO	70.88±0.33**
AEHO	35.60±0.28*

Values are mean ± SEM, (n = 3). Bold text indicates the highest flavonoid content of methanol extract of *Hopea odorata* (MEHO). The different superscripted (*) values have significantly different (*P < 0.05, **P < 0.001) from the other sample in same column.

4. Discussion

The antibacterial effect of the extracts of *Hopea odorata* leaves were analyzed in the disc diffusion technique. This showed that this plant has well antimicrobial activity. In the present investigation, the extracts exhibited better antibacterial activity for *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi* and *Shigella dysenteriae* compared to *Bacillus Megaterium* and *Escherichia coli*. A narrow zone of inhibition was found against *Escherichia coli* compared to others activity.

Methanol extract of *Hopea odorata* leaves exhibited total flavonoid content as 85.98 ± 0.76 mg quercetin/g and this extract also showed highest antibacterial activity against almost all bacteria. On the other hand, EEHO and AEHO contained low flavonoid content and also showed low antibacterial activity compared to MEHO.

Flavonoids are ubiquitous in photosynthesising cells and therefore occur widely in the plant kingdom^[19]. They are found in fruit, vegetables, nuts, seeds, stems and flowers as well as tea, wine^[20], propolis and honey^[21], and represent a common constituent of the human diet^[22]. For centuries, preparations that contain flavonoids as the principal physiologically active constituents have been used by physicians and lay healers in attempts to treat human diseases^[19].

Increasingly, flavonoids are becoming the subject of medical research. They have been reported to possess many useful properties, including anti-inflammatory activity, oestrogenic activity, enzyme inhibition, antimicrobial activity^[19, 22], antiallergic activity, antioxidant activity^[20], vascular activity and cytotoxic antitumor activity^[23]. For a group of compounds of relatively homogeneous structure, the flavonoids inhibit a perplexing number and variety of eukaryotic enzymes and have a tremendously wide range of activities. In the case of enzyme inhibition, this has been postulated to be due to the interaction of enzymes with different parts of the flavonoid molecule, e.g. carbohydrate, phenyl ring, phenol and benzopyrone ring^[19]. Several reviews have been written on the interaction between flavonoids and mammalian cells, including comprehensive articles by Harborne and Williams^[23] and Middleton et al.^[24]. An extensive review on the biochemistry and medical significance of flavonoids has also recently been produced by Havsteen^[25]. So flavonoid cause damage of cell membrane, leading to the inhibition of macromolecular synthesis. Taking into account the *in vivo* safety and their significant antimicrobial potency, which means plant flavonoids, are promising leads for further drug development.

The present studies suggested that flavonoid content responsible for antibacterial activity. Because highest flavonoid containing extract gave highest antibacterial effect and lowest one gave lowest antibacterial activity.

5. Conclusions

This study showed that *Hopea odorata* leaves contained good amount of flavonoid, which exhibited well antibacterial activity. Because highest flavonoid content extract (MEHO) showed highest antibacterial activity against almost all bacteria and lower one (AEHO) showed low antibacterial activity compared with MEHO. So it is suggested that *Hopea odorata* leaves could be considered as source of antimicrobial agent which might be applied in pharmaceutical and cosmetic products.

6. References

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