

Preliminary Phytochemical and Antibacterial studies on Leaf and Bark of *Holigarna grahamii* (Wight) Kurz. (Anacardiaceae)

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ABSTRACT

Holigarna grahamii (Wight) Kurz. is large tree belonging to family anacardiaceae. The bark is smooth and gray in colour, leaves are simple, alternate, glabrous, and midrib is flat. Medicinal plants contribute in human health care system. Most of the plants utilized by village peoples as a folk medicine. Antibacterial activity of the leaves and bark extracts of *Holigarna grahamii* (Wight) Kurz. against pathogenic bacteria like gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) and gram negative (*Escherichia coli* and *Salmonella typhi*, *Proteus vulgaris*) bacteria by in vitro agar well diffusion method. The maximum zone of inhibition of bark extract is observed in *Staphylococcus aureus* (8.33±1.15) while in leaves extract it is observed in *Escherichia coli* (2.66±0.57). The phytochemical screening of leaf and bark extract shows presence of phenols, tannins, alkaloids and reducing sugar.

INTRODUCTION

Medicinal plants has assumed greater importance in recent days, due to the tremendous potential they offer in formulating new drugs against many disease that affect the human kind (Kumar *et al.*2009). Various plants have dual significance firstly, they are promising future food secondly, these medicinal plants, can have some active constituents for future pharmaceutical analysis. This indicates the importance of large number of plants in tribal medicine, which could be of paramount interest for research and drug development and identification of new bioactive compounds. A major part of the population in developing countries still uses traditional folk medicine obtained from plant resources (Srivastava *et al*, 1996). Human being commonly uses the plants to treat common disease from rural practitioners. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Ahmad and Beg 2001). There is insufficient scientific data available to justify the traditional antibacterial potential of this plant; therefore present study is aimed to estimate the therapeutic use of this plant as an antibacterial agent. Since no report found on the antibacterial activity of leaf and bark of *Holigarna grahamii* (Wight) kurz,

present study was conducted to evaluate these property. The many medicinal plant parts are used for the extraction which has a variety of medicinal properties. The different parts used include the root, stem, flower, fruit and twig. While some of these raw drugs are collected in smaller quantities by local communities and folk healers for local used, many other raw drugs are collected in larger quantities and sold at markets as the raw materials for many herbal medicines.

MATERIAL AND METHODS

Collection and identification of plant samples

Plant material was collected from Phonda and Amboli region. These plants were identifying by using relevant literature.

Preparation of Plant Extract

Samples were dried at room temperature for 48 h. Then these plant material were powdered using mechanical grinder. Leaf and bark powder was extracted using Methanol as a solvent. Dried leaves powder 20 gram was weighed and put in a cheese cloth and subjected to extract successively with 200 ml methanol in Soxhlet extractor until the extract was clear. All the extracts were condensed and preserved in refrigerator in air tight bottles until further use.

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Phytochemical screening

The methanolic extract were subjected to qualitative phytochemical screening for the presence or absence of phenols, tannins, saponins, alkaloids, glycosides, reducing sugar and amino acid (Harborne 1998).

Bacterial Strain

In the present investigation bacterial strain were used that are some gram positive bacteria like *Bacillus subtilis*, *Staphylococcus aureus*, and gram negative bacteria *Escherichia coli*, *Salmonella typhi* and *Proteus vulgaris*.

Preparation of media

Weighed accurately 28 gm of hydrated nutrient agar was dissolved in the 1000ml of distilled water. Then these medium was sterilized in autoclave under 15 Lb pressure for 15 minutes. 20ml of this sterilized semisolid nutrient agar medium was poured into pre sterilized glass petriplates. It is carried out under aseptic condition under laminar air flow. Then these plates were cool at room temperature to solidify the medium.

Antibacterial Screening

For determination of antibacterial activity Agar well diffusion method which is described by Perez *et al* (1990). Well of 6mm diameter was prepared with sterilized cork borer. Standard antibiotics Riboflavin served as positive control and Methanol as a negative control. Then these petriplates were inoculated with different bacterial species. These petriplates were incubated at 37 C in incubator for 24 h. Then after 24 h zone of inhibition was measured.

RESULTS AND DISCUSSION

In the present investigation, the phytochemical screening and antibacterial activity of methanolic extract of leaf and stem bark of *Holigarna grahamii* (Wight) Kurz. were done. The phytochemical screening of methanolic extract of leaves as well as bark of *H. grahamii* (Table no 1) shows presence of phenols, tannins, alkaloids and reducing sugar. Pradeep and Saj (2010) were carried out phytochemical and antimicrobial studies of leaf and bark extracts of *Holigarna arnottiana* Hook f. They found out alkaloids, steroids, tannins, phenolic compound, flavonoids, resins, fatty acid, gum from bark and leaves. These compounds may be responsible for the antibacterial activities of the leaf and bark extract. From these it shows similar compound like phenolic, alkaloids and tannins present in both plants may be because of belonging to same genus. Preliminary phytochemical analysis of some important Indian plant species were studied by Kantamerreddi *et. al* (2010). They have taken the methanolic extract of leaf and bark of *Semecarpus anacardium* L. (Anacardiaceae). It shows presence of alkaloids, steroids and terpenoids. Arif *et al.* 2009 were worked on Pharmacognostical studies and evaluation of total phenolic contents of trunk bark of

Spondias mangifera. The bark shows presence of Flavonoids, tannins, xanthoprotein, phenolic. These compounds are also present in bark of *H. grahamii*.

Table. 1: Preliminary phytochemical analysis of leaf and bark of *H. Grahamii* (Wight) Kurz.

Sr. No	Chemical constituents	Leaf Extract (Methanol)	Bark Extract (Methanol)
1	Phenols	+++	+
2	Tannins	+++	++
3	Saponins	-	-
4	Alkaloids	+	+
5	Amino acid	-	-
6	Reducing sugar	+++	-
7	Glycosides	-	-

+ = Slight colour change, ++ = Moderate, +++ = Highly colour change, - = No change

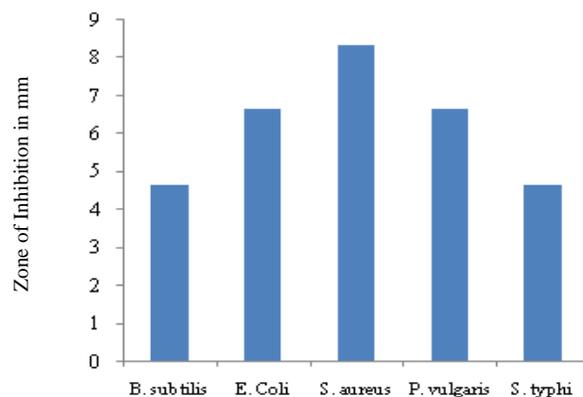


Fig. 1: Effect of Bark extract (Methanolic) of *H. grahamii* on the growth of different Bacterial strains.

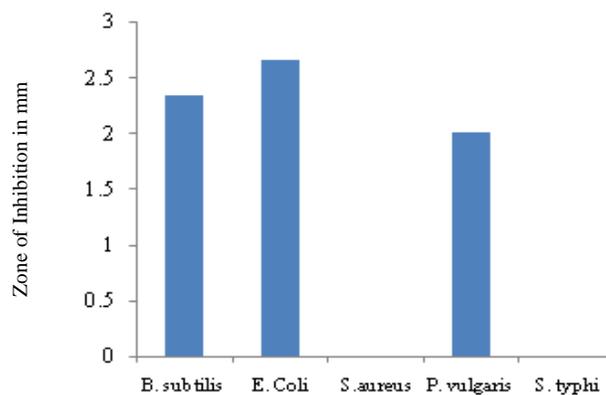


Fig. 2: Effect of Leaf extract (Methanolic) of *H. grahamii* on the growth of different Bacterial strain.

In methanolic extract of bark maximum zone of inhibition exhibit against *Staphylococcus aureus* which is 8.33 ± 1.15 . While the minimum zone of inhibition of bark extract shows against *Bacillus subtilis* and the diameter of zone is 4.66 ± 1.15 . (Table no. 2) Sharma *et. al* (2010) were worked on antimicrobial efficacy of nut oil of *Semecarpus anacardium* (Anacardiaceae) L. f. A marking nut tree. They evaluated antimicrobial activity against gram positive *Bacillus subtilis*, *Staphylococcus aureus* and gram negative *Proteus vulgaris*, *Escherichia Coli* etc. In this *Staphylococcus aureus* showed maximum zone of inhibition that is

16.7±0.80. In present work also shows maximum zone of inhibition in *Staphylococcus aureus*. Bolin and Gogoi (2011) were carried out antibacterial activity of the methanolic extract of stem bark of *Spondias pinnata*, *Moringa oleifera* and *Alstonia scholaris*. It shows significant antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis*. In methanolic extract of leaves of *H. grahamii* the maximum zone of inhibition found in *Escherichia coli* which is 2.66±0.57. While *Proteus vulgaris* showed minimum zone of inhibition and the diameter of the zone is 2.0±1.0.

Table. 2: Zone of Inhibition (mm) of Methanolic extract of Leaves and Bark of *Holigarna grahamii* (Wight) kurz. against some bacterial strains.

Sr. no	Organism	Methanol (Negative)	Riboflavin (Positive)	Bark (mm)	Leaves (mm)
1	<i>Bacillus subtilis</i>	0±0.0	10.33±0.57	4.66±1.15	2.33±0.57
2	<i>Escherichia Coli</i>	0±0.0	7.66±1.15	6.66±1.52	2.66±0.57
3	<i>Staphylococcus aureus</i>	0±0.0	11.33±1.54	8.33±1.15	—
4	<i>Proteus vulgaris</i>	0±0.0	7.33±0.57	6.66±2.51	2.0±1.0
5	<i>Salmonella typhi</i>	0±0.0	8.66±1.15	4.66±1.52	—

Thomas *et. al* (2012) worked on antibacterial and anti inflammatory activities of *Anacardium occidentale* leaves and bark extracts.

From aqueous and ethanolic extract of leaves and bark shows maximum zone of inhibition in *Escherichia coli* which is 12±0.90 and minimum in *Enterococcus faecalis* 7±0.00. In methanolic extract of leaves there is no occurrence of inhibition zone in *Staphylococcus aureus* and *Salmonella typhi*. But in Bark extract of *Staphylococcus aureus* showed maximum zone of inhibition. The zone of inhibition is promising in bark extract as compare to leaves extract. Phytocompounds with antibiotics is required to exploit there potential plant extract in the combination therapy of infectious diseases caused by multidrug resistant organism. The finding of present Investigations support the

traditional knowledge of local users about the selection of plant material as antimicrobial agent and it is a preliminary scientific validation for the use of this plant for antibacterial activity.

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