The aim of this study is to determine the antimicrobial activity and phytochemical constituents of heart wood of *pterocarpus marsupium*. The alcoholic extract of heart wood of *pterocarpus marsupium* was tested against two gram positive (*Enterococci* and *Staphylococcus aureus*) and negative (*Escherichia coli* and *Pseudomonas aeruginosa*) microbial organisms and a fungal strain *Candida albicans*. The heart wood extract was also evaluated for determining the phytochemical constituents. All the microorganisms tested were sensitive to the extract which demonstrated the positive activity of the extract. The phytochemical analysis revealed the presence of saponins, tannins, triterpenes and flavonoids which could also be responsible for the antidiabetic activity of the plant extract.

**KEY WORDS**: *Pterocarpus marsupium*, medicinal plants, active constituents, antimicrobial activity.

**INTRODUCTION**

Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value. Herbal medicine have gained popularity in developing countries due to its
lack of adverse effects (Ali H et al, 2006) when compared to anticonventional anti-diabetic drugs (Marles R et al, 1994). During the past few years some of the new bioactive drugs isolated from plants showed antidiabetic activity with more efficacy than oral hypoglycemic agents used in clinical therapy. It has been pointed out that more than 80% of world population depends on plants to meet their primary health care needs (Atmakuri and Dathi, 2010). The World health Organisation (WHO) which is concerned with global public health has also substantiated the utilization of herbal remedies for the management of diabetes (Bailey CJ et al, 1989). The modern oral hypoglycemic agents produce undesirable side effects. Thus an alternative therapy with fewer side effects is required to treat hypoglycemia. According to a survey conducted by WHO (1998) it has been shown that most of the modern prescription contain plant derived molecules as a base in their drug formulations. Phytochemicals identified from traditional medicinal plants are presenting an exciting opportunity for the development of new types of therapeutics. *Pterocarpus marsupium* roxb (Fabaceae) popularly known as Indian Khino Tree, Malabar Kino Tree. In Karnataka the plant is known as Honne or Kempu hone restricted distribution to southern part of India. It is commonly found in Western Ghats – Tamilnadu, Karnataka and Kerala. The Indian Khino is a moderate sized to large deciduous tree which can grow up to 30 meterstall. Parts of the Khino tree (heart wood, leaves flowers) have long been used for their medicinal properties in Ayurveda. The heartwood is used as an astringent and in the treatment of inflammation and diabetes. It is good for dysentery, cough, diarrhea and grayness of hair. The wood and bark of *pterocarpus marsupium* are known for their antidiabetic activity.

**MATERIALS AND METHODS**

*Preparation of the plant extract* The heart wood of *P.marsupium* was collected locally (Udupi, Karnataka, India). It was dried in open air and finally grounded into fine powder. Finely dried powder was soaked in deionised water for 4-5 hours. Reflux for 2-3 hrs and decanted when it was hot. Filter it using a cotton plug. Concentrate the contents under reduced pressure (evaporate the water) and keep in water bath until it attains a syrupy consistency. Dry and weigh the extract. The dried aqueous extract was packed in air tight container and stored in a desiccator at room temperature.

**Antimicrobial organisms**

2 strains of gram + ve bacteria, *Eutrococci & Staphylococcus aureus* (ATCC 25923) and 2 strains of Gram –ve bacteria Escherichia coli (ATCC 25923) and *pseudomonas aeruginosa* (ATCC) and *Candida albicans* were used as test organism.
Preparation of Inoculum

Stock cultures were maintained at 4°C on slopes of nutrients agar. Active cultures for experiments were prepared by transferring a loopful from the stock cultures to test tubes of peptone water for bacterial isolates and Saboraunds broth for candida. The tubes were incubated without agitation for 24 hrs at 37°C. The cultures were diluted with fresh peptone water to achieve optical densities to 2.0 x 10^6 colony forming units (CFU/ml). (Richard Schwalbe et al, 2007).

Antimicrobial Activity

The Agar diffusion (punch well) method was used to screen the antimicrobial activity. In vitro antimicrobial activity was screened by using Mueller Hinton Agar (MHA) (dehydrated medium) obtained from Himedia (Mumbai). The test inoculums swabbed uniformly on to the MHA plates and wells of diameter 6mm were punched out in each plate. Ethanol extract of 100 µl were loaded into the wells. The loaded wells were allowed to diffuse for 5 min and the plates were placed upright for incubation at 37°C for 24hrs. At the end of incubation, inhibition zones formed around the wells were measured with transparent ruler in millimetres. These studies were performed in triplicate and mean values were recorded.

Phytochemical screening methods

Test for Saponins

One gram of extract was boiled with 10 ml of water for 4 minutes. The mixture was cooled, mixed vigorously and left for few minutes. The formation of frothing indicates the presence of saponins. (Kapoor et al, 1969)

Test for tannins

Two ml of the extract was added with 2 ml of ferric chloride (1%). Color development from red-brown to blue black indicates the presence of tannins. (Kapoor et al, 1969)

Test for Triterpenes

The extract (1.0 g) was mixed with chloroform and warmed at 55°C for 30 minutes. To this added 1.0 ml of concentrated sulphuric acid and mixed well. The appearance of a reddish brown color indicates the presence of triterpenes. (Harborne et al, 1973)
Test for Flavonoids

One gram of the extract was boiled with 10 ml of ethyl acetate over a steam bath for three minutes. The 4.0 ml filtrate was mixed with 1 ml of dilute ammonia solution and a yellow precipitate indicates the presence of flavonoids. (Kapoor et al, 1969)

RESULTS AND DISCUSSION

Medicinal plants are known to have beneficial therapeutic effect documented in traditional Indian system of medicine. The increase of antibiotic resistance by the pathogenic microorganism to conventional drugs has made us search for new, efficient & cost effective drug for the control of infectious diseases. Many reports showed that medicinal plants constitute a great source for the isolation of active drugs for the control of pathogenic organisms. Several reports are available in support of antibacterial activity of several phytochemicals present in plant extracts. In our present study the result demonstrated that all the three concentrations (25,50,100µg/ml)of the extract showed antimicrobial action.. However, it was found that *Pterocarpus marsupium* showed high sensitivity with pseudomonas in 100µgm/ml when compared to 50 and 25µg of the extract against both gram positive and gram negative test organism. When the extract was evaluated at all the three concentrations against *Candida albicans* it did not show any antifungal efficacy.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Zone of inhibition (mm)</th>
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<tr>
<td></td>
<td>100 µg/ml</td>
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<tr>
<td><em>Pseudomonas</em></td>
<td>24</td>
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<tr>
<td><em>Escheria coli</em></td>
<td>8</td>
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<tr>
<td><em>Enterococci</em></td>
<td>14</td>
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<tr>
<td><em>Candida albicans</em></td>
<td>R</td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
<td>22</td>
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*Phytochemical analysis*

The phytochemical assay was carried out as described by (Kapoor L.1969) and Harbone JB (1998). This analysis was done to detect the presence or absence of phytochemicals like alkaloids,saponins,triterpenes,flavonoids and sterols. Phytochemical screening of alcoholic extract revealed the presence of saponins, triterpenes, tannins and flavonoids which suggests that the plant extract possess free radical scavenging action in addition to antimicrobial action. Saponins present in the plant are suggestive of their anti-hyperlipidemic properties. Flavonoid which is known to have antioxidant properties tries to regenerate the damaged beta cells of pancreas. The antioxidant activity of the phenolics, tannins and flavonoid compounds are attributed to its redox properties which can act as reducing agents, hydrogen donators and
single oxygen quenchers (Gulcin I et al, 2007). Polyphenolics having hydroxyl groups are very important plant constituents which can protect from oxidative stress. (Jing et al, 2010).

CONCLUSION
Crude extracts of Pterocarpus marsupium showed an effective antimicrobial efficacy against gram +ve and -ve organisms. Presence of phytochemical constituents could likely be responsible for the antibacterial activity. However further studies are required to establish that the plant extracts could form effective antimicrobial therapy against common bacterial diseases.

REFERENCES