Phytochemical Screening and Evaluation of Antimicrobial Activity of Semecarpus anacardium Nuts

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Abstract - Many herbal remedies individually or in combination have been recommended in various medical treatments for the cure of different diseases. *Semecarpus anacardium* (SA) Linn (Family: Anacardiaceae), is a plant well known for its medicinal value in ayurvedic and siddha system of medicine. The *Semecarpus anacardium* nuts are used for various medicinal and pharmacological purposes from ancient period. The petroleum ether nut extract of *Semecarpus anacardium* were screened for phytochemicals and revealed the presence of Phenols, Triterepenoids, Steroids, Alkaloids, Flavonoids, Saponins, Tannins and Anthraquinones. The petroleum ether nut extract of *Semecarpus anacardium* exhibited antibacterial property against gram positive bacteria and gram negative bacteria by Agar well method. The nut extract showed inhibitory activity against test organisms like *Escherichia coli* (19 mm), *Micrococcus luteus* (23 mm), *Salmonella typhi* (26mm), *Bacillus subtilis* (14 mm) and *Klebsiella pneumonia* (22 mm). The antibacterial activity of nut extract of *Semecarpus anacardium* is due to Petroleum Ether extractable compounds.

Keywords- *Semecarpus anacardium*, Phytochemical screening, antibacterial activity.

I. INTRODUCTION

The exploitation of plants by man for the treatment of diseases has been in practice for a very long time. Herbal drug constitutes a major part in all the traditional system of medicines [1]. Screening of compounds obtained from plants for their pharmacological assay has indeed been the vast source of innumerable therapeutic agents representing molecular diversity engineered by nature. It is therefore necessary and urgent to fight against emerging and reemerging infectious diseases. Further, newer strains are being continuously discovered which are refractory to the current arsenal of drugs [2]. The World Health Organisation (WHO) estimated that 80% of the population of developing countries rely on traditional medicine mostly plant drugs, for their primary health care needs. Medicinal plants are being natural, non-narcotic, having no side effect. Demand for medicinal plants is increasing in both developing and developed countries. Over the past few decades; there has been much interest in natural materials as source of new antibacterial agents. Different extracts from traditional medicinal plants have been tested. Many reports show the effectiveness of traditional herbs against micro organisms as a result plants have become one of the bases of modern medicine [3]. Natural product of higher plants may give a new source of antibacterial agents with possibly a novel mechanism of action. The selection of crude plant extract for screening the antibacterial activity has the potential of being more successful in the initial steps than screening of pure compounds [4].

*Semecarpus anacardium* (SA) linn (Family: Anacardiaceae) is distributed in sub-Himalayan region, tropical and central parts of India. The nut is commonly known as ‘marking nut’ and in the vernacular as ‘Ballataka’ or ‘Bhilwa’. Detoxified nut of *Semecarpus anacardium* were incorporated in prescription for toxic conditions, obstinate skin diseases, tumours, malignant growth, fevers, haemoptysis, excessive menstruation, vaginal discharge, deficient lactation, constipation, intestinal parasites. The nuts are also used for variety of disorders in Ayurveda. It has been used therapeutically in neurological disorders, ulcers [5]. Many compounds mainly biflavonoids, phenolics, bhilawanols, sterols, Anacardic acid, and glycosides have been identified as constituents of *S. anacardium* nut extract. Studies have also reported that the drug has anti-inflammatory, antiarthritic, anthelmentic, antioxidative and anticancer activity [6, 7]. Some organisms have developed resistance to the existing antibiotics, therefore the development of bacterial resistance to the currently available antibiotics has necessitated the research for new antibacterial agents [8]. As *Semecarpus anacardium* nuts has lot of medicinal significance, the present work was aimed for Phytochemical screening of
Phytochemical Screening and Evaluation of Antimicrobial Activity of Semecarpus anacardium Nuts

II. MATERIALS AND METHODS:

A. Collection and identification of plant:

The plant Semecarpus anacardium nuts were collected from the University of Agriculture Science (UAS), Dharwad. The nuts of Semecarpus anacardium were taxonomically identified and authenticated by Dr. B.D. Huddar, Professor and Head Dept. of Botany, S.K Arts College & H.S.K. Science Institute Vidyanagar, Hubli. The plant sample was deposited at Department of Biotechnology, B.V. Bhoomaraddi College of Engineering & Technology.

B. Preparation of Plant Nut Extract:

The whole plant nuts were cleaned and shade dried for 10-15 days. The dried nuts were pulverized by an electrical blender and nut paste was obtained. About 30-40 g of the nut paste was subjected for extraction with 400 ml of Petroleum ether solvent by Soxhlet apparatus for 24 hrs. Constant heat of 50 - 60 °C was provided by Mantox heater of Soxhlet for recycling the solvent. The extract was concentrated using Rotary evaporator (Scietyk, MODEL:RE 300) at 60 °C for 20 min at a speed of 5m/s. The concentrated extract was kept in refrigerator at 4 °C for further use.

C. Preliminary screening of Phytochemicals:

The different qualitative tests were performed for establishing profile of given extract for its chemical composition. The following tests were carried out on extracts to detect various phytoconstituents present in Semecarpus anacardium plant nuts extract.

a. Detection Alkaloids [9]

Mayer’s Test: Extract was treated with Mayer’s reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner’s Test: Extract was treated with Wagner’s reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff’s Test: Extract was treated with Dragendroff’s reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager’s Test: Extract was treated with Hager’s reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

b. Detection of Phenols [10]

Bromine water test: Test solution was treated with few milliliters of bromine water. Formation of yellow precipitate indicates presence of Phenols.

Ferric chloride test: Test solution gives blue green colour with ferric chloride.

c. Detection of Saponins

Emulsion test: 1 ml of the extract filtrate was added to few drops of olive oil. The mixture was shaken and observed for the formation of emulsion.

Frothing test: 1 ml of the extract filtrate was diluted with 4 ml of distilled water. The mixture was shaken vigorously and then observed on standing for a stable froth.

d. Detection Steroids and Triterpenoids

Libermann-Buchard test: Extract was treated with few drops of acetic anhydride, boiled and cooled, conc. Sulphuric acid was added from the sides of the test tube. Formation of a brown ring at the junction of two layers and the upper layer turns green which shows the presence of Steroids and formation of deep red colour indicates the presence of Triterpenoids.

Salkowski test: Treat extract in Chloroform with few drops of cone. Sulphuric acid, shake well and allow standing for some time, red colour appears at the lower layer indicates the presence of Steroids and formation of yellow coloured lower layer indicates the presence of Triterpenoids.

e. Detection of Tannins

Lead sub-acetate test: 1 ml of the filtrate was added to 3 drops of the lead sub-acetate solution. A cream gelatinous precipitate indicates the presence of tannins.

Ferric chloride test: 1 ml of the filtrate was diluted with distilled water and added with 2 drops of ferric chloride. A transient greenish to black colour indicates the presence of tannins.
f. Detection of Flavonoids

Shinoda test (Magnesium Hydrochloride reduction test)

To the test Solution, add few fragments of Magnesium ribbon and add concentrated Hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.

Alkaline reagent test:

To the test solution add few drops of sodium hydroxide solution; formation of an intense yellow colour, which turns to Colourless on addition of few drops of dil. acid, indicates presence of Flavonoids.

Ammonium chloride test:

A quantity (4 ml) each of the filtrates was shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration at the ammonia layer indicates the presence of Flavonoids.

Aluminium chloride test:

A quantity (4 ml) each of the filtrates was shaken with 1 ml of 1% aluminium chloride solution and observed for light yellow coloration. A yellow precipitate indicates the presence of Flavonoids.

g. Detection of Anthraquinones

1. Dilute sulphuric acid (5 ml) was added to 0.1 g of the test extract in a test tube and boiled for 15 min in a water bath. It was then cooled and neutralized with 20% potassium hydroxide solution. A mixture, 10 ml of equal parts of Fehling’s solution A and B was added and boiled for 5 min. A more dense red precipitate indicates the presence of glycoside.

2. About 0.5 ml of extract was taken and subjected to the following tests. 1 ml of glacial acetic acid containing traces of ferric chloride and 1ml of concentrated sulphuric acid were added to the extract and observed for the formation of the reddish brown colouration at the junction of two layers and the upper layer turned bluish green shows presence of Glycosides.

D. Antibacterial activity

a. Micro organisms used:

For the present study pure Bacterial cultures were used from our laboratory. Some of the Pure Bacterial cultures were procured from National Collection of Industrial Microorganisms (NCIM), Pune and Microbial Type Culture Collection (MTCC), Chandigarah. The bacterial Culture namely, *Salmonella typhi*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia* were used as test organisms to determine the antibacterial activity. All bacterial staines were sub cultured frequently every 15 days and maintained on nutrient agar slants.

B. Agar well method:

Antibacterial activity of all plant extracts were tested by modified Agar well method. Inoculum suspension was spread over the agar plates using sterile L-shaped glass rod. Well of 0.5 cm in diameter was made in inoculated media plate and 150 µl extracts of *Semecarpus anacardium* nuts were aseptically filled into the well. The plates were placed at room temperature for an hour to allow diffusion of extract into the agar. Then the plates were incubated for 24 hrs at 37°c. The results were recorded by measuring the diameter of inhibitory zone using a transparent meter rule at the end of 24-48 hrs.

c. Broth dilution method:

Quantitative analysis of Antibacterial effect of Petroleum Ether nut extract of *Semecarpus anacardium* were determined by Broth dilution method. 1ml of plant extracts was added to 10 ml nutrient broth in 20 ml test tubes. The tubes were then inoculated with appropriate test bacteria and incubated at 30°c ±1 in an orbital shaker (Scigenic Bioteck Pvt Ltd) at 100 rpm. The inhibition of Bacterial growth was determined spectrophotometrically (ELICO, MODEL:SL159) by measuring the absorbance at 625 nm at various time interval [11].

III. RESULTS

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites. The Petroleum ether nut extract of *Semecarpus anacardium* have revealed the presence of various metabolites like alkaloids, Flavonoids, glycosoids, phenols, Saponins, steroids, Triterpenoids and Anthraquinones. Thus the preliminary tests may be useful in detection of the bioactive principles and subsequently may be lead to the drug discovery and development. The Phytochemical analysis of Petroleum ether nut extract of *Semecarpus anacardium* is as shown in Table 1. The antimicrobial properties of substances are desirable tools in the control of undesirable microorganisms especially in the treatment of infectious diseases and in the food spoilage. The active components usually interfere with growth and metabolism of microorganisms in a negative manner [12]. Several phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens. Many plants contain non-toxic glycosides that can get hydrolyzed to release phenolics that are toxic to microbial pathogens. Therefore, the compounds detected in the nuts of S. anacardium are responsible for the antibacterial activity [13, 14]. The petroleum ether solvent extract of *Semecarpus anacardium* nuts showed antibacterial
activity against all the test organisms shown in Table 2. Ampicillin was used as standard antibiotic. The Zone of inhibition for different Microorganisms of petroleum ether nut extract of *Semecarpus anacardium* as shown in fig. 1, 2, 3, 4 and 5. In broth dilution, the petroleum ether nut extract of *S.anacardium* showed more inhibitory effect on *Salmonella typhi*. It also showed very good inhibitory activity against tested organisms at different time intervals as shown in fig. 6.

Table 1. Phytochemicals analysis of Petroleum ether nut extract of *Semecarpus anacardium*

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Tests</th>
<th>Petroleum ether nut extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>Alkaloids</strong></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>a. Mayer’s test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>b. Dragendorff’s test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>c. Wagner’s test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>d. Hager’s test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>e. Tannic acid test</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td><strong>Saponins</strong></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>a. Emulsion test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>b. Frothing test</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td><strong>Steroids &amp; Triterpenoids</strong></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>a. Libermann- Buchard test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>b. Salkowski test</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td><strong>Tannins</strong></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>a. Lead sub-acetate test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>b. Ferric chloride test</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td><strong>Flavonoids</strong></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>a. Shinoda test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>b. Alkaline reagent test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>c. Ammonium test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>d. Aluminium chloride test</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td><strong>Anthraquinones</strong></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>a. Borntrager’s Test</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td><strong>Phenols</strong></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>a. Bromine water test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>b. Ferric chloride test</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2. Zone of Inhibition in mm

<table>
<thead>
<tr>
<th>Micro organisms</th>
<th>Petroleum ether nut extract (150µl)</th>
<th>Ampicillin (standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E coli</em></td>
<td>19</td>
<td>06</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>14</td>
<td>11.4</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td>23</td>
<td>10</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>26</td>
<td>6.5</td>
</tr>
</tbody>
</table>

**III. DISCUSSIONS**

The increase of antibiotic resistance of microorganism to conventional drugs has necessitated the search for new efficient and cost effective ways for the control of infectious diseases. Many reports show the effectiveness of traditional herbs against microorganisms as a result plants have become one of the bases of modern medicine [15].

Analysis of Petroleum ether solvent nut extract of the *S.anacardium* plant demonstrated the presence of phytochemicals like terpenes, Flavonoids, Phenolics, Saponins, Alkaloids and glycosides. These phytochemicals posses anti microbial activity. The plants are rich in a wide variety of secondary metabolites which were found to have *in vitro* antimicrobial properties [16]. The antimicrobial activity found in the plant extracts have been attributed to some of the secondary metabolite [17, 18]. The presence of phenolic compounds in the extract may attribute antibacterial activity. Phenolic compounds are thought to be toxic to micro organisms, inhibiting the enzymes which are essential for the growth of microorganism.

The antimicrobial activities of phenolic compounds may involve multiple modes of action for eg, oils degrade the cell wall, interact with the composition and disrupt cytoplasmic membrane [19], damage membrane protein, interfere with membrane integrated Enzymes [20],cause leakage of cellular components, coagulate cytoplasm, deplete the proton motive force, change fatty acid and phospholipids constituents, impair enzymatic mechanism for energy production and metabolism, alter nutrient uptake and electron transport.

The antibacterial activity of *S. anacardium* and nature of active principles present in the extracts of this plant is demonstrated for the first time against the pathogenic bacteria. These results suggest the possible exploitation of this plant in the management of the infectious diseases. Further purification of the extract may yield a novel antibacterial drug. Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant extract for their antimicrobial activity may provide some more new antimicrobial substances.

From our results in broth dilution technique, the petroleum ether nut extract of *S.anacardium* has successfully controlled the growth of all microorganisms which were tested. In the Broth dilution method antibacterial assay of Petroleum ether nut extract of *S. anacardium* showed more inhibitory effect on *Salmonella typhi*. It also showed very good inhibitory activity against tested organisms at different time intervals as shown in fig. 6.
extract of *S. anacardium* was most effective against Gram -ve strains (*Salmonella typhi*, *Klebsiella pneumoniae* and *E. coli*) compared to Gram +ve strains (*Micrococcus luteus*, *Streptococcus aureus* and *Bacillus subtilis*). These results are in agreement with earlier studies with different plants as reported by previous workers [21, 22].

Fig 1: Zone of inhibition for *Micrococcus luteus*

Fig 2: Zone of inhibition for *Salmonella typhi*

Fig 3: Zone of inhibition for *Bacillus subtilis*

Fig 4: Zone of inhibition for *Klebsiella pneumoniae*

Fig 5: *E. coli* with standard Ampicillin

Fig 6: Effect of petroleum ether nuts extract of *S. anacardium* on pathogenic gram -ve bacteria in broth dilution method.
IV. CONCLUSION

Our results support the use of these plants as traditional medicine and suggest that some of the plant extracts possess compounds with good antibacterial properties that can be used as antimicrobial agents in the search of new drugs. *Semecarpus anacardium* is significantly active against *Salmonella typhi*. The preliminary phytochemical analysis of *Semecarpus anacardium* revealed the presence of Triterpenoids, steroids, Anthraquinones and phenols which have contributed to effective antibacterial activities.

V. ACKNOWLEDGMENT

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