INTRODUCTION

Azadirachta indica (Meliaceae) commonly known as neem is native to India and naturalized in most of tropical and subtropical countries is of great medicinal value and distributed wide spread in the world. The Chemical constituents contain many biologically active compounds that can be extracted from neem, including alkaloids, flavonoids, triterpenoids, phenolic compounds, Carotenoids, steroids and ketones. Azadirachtin is actually a mixture of seven isomeric compounds labeled as azadirachtin A-G and azadirachtin E is more effective (Verkerk et al., 1993). Other compounds that have a biological activity are salannin, volatile oils, meliantriol and nimbin (Jacobson et al., 1990; Ahana et al., 2005). Neem leaf is effective in treating eczema, ringworm, acne, anti-inflammatory, antihyperglycemic properties and it is used to heal chronic wounds, diabetic food and gangrene developing conditions. It is believed to remove toxins from the body, neutralize free radicals and purify the blood. It is used as anticancer agent and it has hepato-renal protective activity and hypolipidemic effects (Fitoterapia part I and part II).

Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases. Medicinal plants are a rich source of antimicrobial agents (Mahesh and Satish et al., 2008). Almost every part of the tree is bitter and finds application in indigenous medicine. Neem extract has been reported to have antidiabetic, antibacterial and antiviral activity (Kirtikar and Basu, 1987). Almost every part of the tree has been in use since ancient times to treat a number of human ailments and also as a household pesticide. The extract from bark, leaves, fruits and root have been used to control leprosy, intestinal helminthiasis and respiratory disorders in children (Chattopadhyay et al., 1993). Flavonoids, flavonoglycosides, dihydrochalocones, tannins and others are also important constituents of bark, leaves, fruits and flowers of neem. The biological activities and medicinal properties of neem have recently been reported (Venugopal and Venugopal, 1994).

Natural drugs have been a part of the evolution of human, healthcare for thousands of years. Nowadays nearly 88% of the global populations turn to plant derived medicines as their first line of defence for maintaining health and compacting diseases. One hundred and nineteen secondary plant metabolites derived from plants are used globally as drugs, 15% of all angiosperms have been investigated chemically and of that 74% of pharmacologically active plant derived components were discovered (Raja and Ramanathan, 2009). Plants are rich in...
a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc. which have been found in vitro to have medicinal properties. Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds (Biswa and Chattopadhyay 2002). Phytochemicals from medicinal plants serve as lead compounds in antimicrobial discovery (Chakravarthy et al., 1985; Ebi et al., 2000; and Cohen et al., 2002).

MATERIALS AND METHODS

Collection of plant materials:

The experiment was conducted in the year 2011 in the college laboratory. Leaves were collected from the Azadirachta indica tree in the college campus. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and to clean the leaves thoroughly and dried.

Preparation of leaf extracts:

20-30 grams of fresh leaves were boiled with 200 mL of solvent for 1 hour. The extract was filtered using Whatman filter paper No. 1 and then concentrated in vacuum at 40°-50°C using a rotary evaporator. Evaporation of solvent in the rotary evaporator affords a crude extract of the soluble components and these extracts were subjected to the qualitative phytochemical analysis and antibacterial studies.

Phytochemical Analysis:

The extracts were analyzed by the following procedures (Talukdar and Choudhary 2010). To test for the presence of the alkaloids, saponins, tannins, Terpenoids, flavonoids, glycosides, volatile oils and reducing sugars

Saponins:

Saponins were detected using the froth test. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5 ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Tannins:

To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue color is observed for gallic tannins and green color indicates for catecholic tannins.

Reducing Sugars

To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling’s solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugars.

Glycosides:

25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%NaOH, then 5ml of Fehling solution added. Glycosides are indicated by a brick red precipitate.

Alkaloids:

2ml of extract was measured in a test tube to which picric acid solution was added. An orange coloration indicated the presence of alkaloids.

Flavonoids:

4ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.

Volatile oils:

2ml of extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl. A white precipitate is formed if volatile oils are present.

Terpenoids:

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid.

Ethanol Extract:

Azadirachta indica leaves (100 g) were ground into fine powders (Himal Pauel Chhetri et al., 2008) using a stainless-steel grinder, and deep in 100% ethanol (200 mL) for overnight. The ethanol fraction was separated using sterile muslin cloth and filter through sterile Whatman filter paper (no. 02). The filtered extract was concentrated by a rotary film evaporator.

Acetone Extract:

For preparation of Acetone extract ground plant sample (100 g) was added in Acetone respectively (200ml each case) and left for overnight at room temperature (Puri et al., 1995). The extracts were separated using sterile muslin cloth and filter through sterile Whatman filter paper (no. 02).

Methanol Extract:

Ten grams of dried plant material was extracted with 100 ml of methanol kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume (17). It was stored at 4°C in airtight bottles for further studies.

Source of microorganisms:

The organisms used were Escherichia Coli, Pseudomonas aeroginosa, Staphylococcus aureus and Salmonella typhi. The organisms were obtained from the Microbial Lab of Department of Microbiology, A.V. C. College, Munnampandal, Mayiladuthurai, Tamilnadu, India.

Determination of Antibacterial Activity:

The antibacterial activity of the leaf extracts was determined using agar well diffusion method by following the known procedure. Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells were punched in the agar and filled with plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 37°C for 18 hours and the antibacterial activity was assessed by measuring the diameter of the zone of inhibition. The antibacterial potential of the different extracts was evaluated by comparing their zones of inhibition.

RESULT

The antibacterial activity of Acetone, Ethanol and
Table 1: Antibacterial activity of Acetone, Ethanol and Methanol extract of *Azadiracta indica* medicinal plants against human pathogens.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Extracts</th>
<th>Zone of inhibition (mm)</th>
<th>Escherichia Coli</th>
<th>Pseudomonas aeruginosa</th>
<th>Staphylococcus aureus</th>
<th>Salmonella typhi</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Azadiracta indica</em></td>
<td>Acetone</td>
<td>17</td>
<td>15</td>
<td>18</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>16</td>
<td>17</td>
<td>12</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>24</td>
<td>20</td>
<td>19</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>


Table 2: Qualitative Phytochemical Analyses of Acetone, Ethanol and Methanol extracts of *Azadiracta indica* Leaf.

<table>
<thead>
<tr>
<th>Solvents used for extraction</th>
<th>Alkaloid</th>
<th>Reducing sugar</th>
<th>Flavonoid</th>
<th>Saponin</th>
<th>Tannin</th>
<th>Volatile oil</th>
<th>Glycoside</th>
<th>Terpenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Methanol</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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</tr>
</tbody>
</table>

 (+) indicates presence while (−) indicates the absence of the components

Methanol extracts was investigated using agar well diffusion method, against the selected human pathogens such as *Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhii*. All the examined extract showed varying degrees of antibacterial activities against the pathogens. The phytochemical test was done to find the presence of active chemical constituents such as glycosides, alkaloids, tannins, flavonoids, terpenoids, saponin, reducing sugar and volatile oil.

Table-1 showed the antibacterial activity of Ethanol extract of *Azadiracta indica* showed maximum zone of inhibition (30mm) against *Salmonella typhii*, followed by *Escherichia Coli* (24mm), *Pseudomonas aeruginosa* (20mm) and *Staphylococcus aureus* (19mm). The antibacterial activity of Acetone extract of *Azadiracta indica* showed maximum zone of inhibition (18mm) against *Staphylococcus aureus*, followed by *Escherichia Coli* (17mm), *Salmonella typhii* (16mm) and *Pseudomonas aeruginosa* (15mm). The antibacterial activity of Methanol extract of *Azadiracta indica* showed maximum zone of inhibition (20mm) against *Salmonella typhii*, followed by *Pseudomonas aeruginosa* (17mm), *Escherichia Coli* (16mm) and *Staphylococcus aureus* (12mm).

The phytochemical analysis of plant extracts using Acetone, Ethanol and Methanol was showed in Table-2. From the phytochemical analysis catecholic reducing sugar were found in *Azadiracta indica* in the solvents such as Acetone, Ethanol and Methanol. The Ethanol extract of *Azadiracta indica* showed the presence of flavonoids, saponins, tannin, reducing sugar were found in presence of Ethanol extract. Reducing sugar, glycosides were observed only in Acetone extract of *Azadiracta indica*. In plant all extracts found glycosides except in Ethanol extract of *Azadiracta indica*. Saponin were observed in the Acetone and Ethanol extract of *Azadiracta indica*. Terpenoids were observed only Methanol extract of *Azadiracta indica*. The Acetone, Ethanol and Methanol all extract of *Azadiracta indica* showed the absence of alkaloid and volatile oil.

**DISCUSSION**

The findings of the preliminary Phytochemical investigations and the results of antibacterial activity were depicted in the respective Tables. The preliminary phytochemical tests performed were of qualitative type and from the phytochemical investigations it was observed that alkaldoids, tannins, flavonoids, terpenoids, saponins Glycoside and compounds reducing were present in the extracts.

The ethanol, chloroform and aqueous extract showed considerable activity against *Salmonella typhii*. The ethanol extract was more active than the standard against *Salmonella typhii*. Previous study conducted by (Ben Gueddeur et al., 2002) suggests that the essential oil of *O. majorana* poses antibacterial activity. The work conducted by (Farooqi and Sreeeramu, 2004) reveals that the leaves of marjoram have antibacterial activity against *Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhii*. Similarly antibacterial activity of ethanol, chloroform and water extract of *Marrubium vulgare*, was further assessed against, *Salmonella typhii*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, were recorded (Al-Bakri et al., 2006).

The presence of these phytochemical components may be responsible for the observed antimicrobial activity of the plant leaf extract. This findings conforms to the report of (Anyanwu and Dawet, 2005) in which similar constituents was found to exhibits antiprotozoal and antibacterial activities. Flavonoid has also been reported to have greater potential benefit to human Health (Jouad et al., 2001).

Iraman khan et al., 2010 studied that phytochemical analysis of *Azadiracta indica* leaves by using different solvent such as Petroleum ether, chloroform, methanol show the presence of triterpenes, glycosides and fatty acids. Other phytochemicals studied in this analysis were absent in all extract of leaves. Antibacterial activity of *Azadiracta indica* was analyzed by previous workers showed that the chloroform extract of leaves possess significant activity, than petroleum ether and methanol extracts. Early studies proved ethanol as the most efficient solvent for extracting broad spectrum of antibacterial compounds from plants.

Himal paudel chhetri et al., 2008 reported that the ethanolic extract of *Azadiracta indica* whole plant shows presence of flavonoids and tannins only. Similarly the extract of *Azadiracta indica* is active against *E.coli* followed by *Staphylococcus aureus*. Earlier observation done by (Srinivasan et al., 2001) also showed the antifungal and antibacterial activity of *A. indica*. 
The methanolic extract of bulbs of *Allium cepa* showed pronounced activity (23mm) against *Bacillus subtilis* and *Pseudomonas aeruginosa*, high activity (20mm) against *Proteus vulgaris*, while inactive against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. The onion bulbs contain numerous organic sulfur compounds, including trans-S-(1-propenyl) cysteine sulfoxide, S-methyl–cysteine sulfoxide, S–propylcysteine sulfoxide and cycloallilin; flavonoids; phenolic acids; sterols including cholesterol, stigma sterol, b-sitosterol; saponins; sugars and a trace of volatile oil composed mainly of sulfur compounds. Although *Allium, Azadirachta* and *Aloe* extracts did not show any activity against *Staphylococcus aureus* (Nima, and Mossa, 1983; and Enkeblia, N.; Dahmouni, S, et al., 2005).

Kumar et al., 2006 studied the antibacterial of dichloromethane: methanol (1:1 v/v) extracts of *Vitex negundo* against different bacterial strains. Their finding conclude that none of the micro organisms including the bacterial strains like *B.subtilis, S.aureus, S.epidermidis, E.coli, and P.aeruginosa* were inhibited by dichloromethane: methanol extracts.

Ahmad et al., 1998 studied the antibacterial activity of the *V.negundo* while plant of hexane, alcoholic and aqueous extracts against *B.subtilis, E.coli, Proteus vulgaris, S.typhimurium, P.aeruginosa* and *S.aureus* had no activity. (Valasraj et al., 1997) studied the antibacterial activity of ethanol extracts of *V.negundo* leaf using agar dilution method against four bacteria *B.subtilis, S.epidermidis, E.coli* and *P.aeruginosa*.

Panda et al., 2009 studied the antibacterial activity of *V. negundo* on bark and leaf of petroleum ether, chloroform, methanol and aqueous extracts against *B.subtilis, S.aureus, S.epidermidis, S.typhimurium, P.aeruginosa, V.cholerae, and V.alginolyteus* had little activity but inhibition was measured including disc and cup that measures 6mm indicates low activity moreover less concentration of extract was taken which dose not give accuracy of results.

The negative results obtained against Gram-negative bacteria were not unexpected since this class of bacteria is usually more resistant than Gram-positive bacteria (Tomas-Barberan et al., 1988). Similar results were also obtained from agar cup method. *E. coli* was completely inhibited by all the extracts of both leaf and bark. *S. aureus* was the second most inhibited bacteria with most of the extracts. Ethanol and methanol extracts of the leaves were most active inhibiting agent against both Gram-positive and Gram-negative bacteria. On other hand petroleum ether and chloroform extracts had better antibacterial activity against all Gram-positive bacteria.

However, *S. epidermidis* and *B. subtilis* were inhibited completely by petroleum ether and chloroform extracts of bark as well as ethanol and methanol extracts of leaves. Infection caused by *P. aeruginosa* are among the most difficult to treat with conventional antibiotics (Levison Jawetz et al., 1992). The growth of *P.aeruginosa* was partially inhibited by petroleum ether and ethanol extracts of leaves and bark and moderately inhibited by chloroform, methanol and aqueous extracts. So the plant *V. negundo* can be used as a source which could yield drugs that could improve the treatment of infection caused by this organism. *B. subtilis* is the common bacteria found in most natural environments including soil, water plant and animal tissues.

Napoleone et al., 2009 also reported Enterobacter spp, *S.aureus, P.aeruginosa, S.typhi* and *E.coli* to be sensitive to ethanol, chloroform and aqueous extract of *Moringa olifera* leaf at concentration of 200 mg/1. phytochemical analysis were similar report of our results. Maluventhan vij et al., 2010 studied that ethanol, chloroform and aqueous extract of *Cardiospernum halicacabum* leaves shows the presence of flavonoids, tannins, steroids and glycosides, which were similar to our results. Antibacterial activity of *Cardiospernum halicacabum* was studied by same workers reported that ethanol extract was active against *Streptococcus aureus* followed by *Salmonella typhi, E. coli* & *P. aeruginosa*. It is also related to our results.

Results obtained from this study, indicate that the plant extracts showed the strongest antibacterial activity than the commercially available antibiotics. For example, Ciprofloxacin showed the maximum zone of inhibition (34mm) against *Streptococcus sp*. but the methanol extract of *Eucalyptus* (*Eucalyptus globulus*) and the methanol extract of *Butterfly Pea* (*Clitoria ternatea*) showed the maximum zone of inhibition (42mm and 36mm) against *Streptococcus sp*.

The phytochemical analysis showed the presence of tannins, glycosides, flavonoids, reducing sugars and saponin, were present in some of the plant extracts.

CONCLUSION

It may be concluded from this study that *Azadirachta indica* leaf extract has antibacterial activity against dental pathogens. It is expected that using natural products as therapeutic agents will probably not elicit resistance in microorganisms. This can explain the rationale for the use of the plant in treating infections in traditional medicine. The plant could be a veritable and cheaper substitute for conventional drugs since the plant is easily obtainable and the extract can easily be made via a simple process of maceration or infusion. It is essential that research should continue to isolate and purify the active components of this natural herb and use in experimental animals.

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