INTRODUCTION

Adhatodavasica is a popular plant in Ayurvedic and Unani systems of medicine to treat different ailments as herbal remedy (Yusuf et al., 2016). Tropical and sub-tropical parts of India are distributed by Adhatodavasica (Shipla et al., 2014). Plant has enormous importance due to their nutritive value and persist to be a major source of medicines as they have been found throughout human history. The digestive enzyme trypsin is found to be activated by leaves of Adhatodavasica. An extract of the leaves demonstrate significant anti-fungal activity against ringworms. It is a small evergreen, sub-herbaceous bush which grows commonly in open plains, especially in the lower Himalayas, India, Sri Lanka, Burma and Malaysia (Santosh et al., 2014). Adhatodavasica is used to control pain, inflammation and other related diseases. Treatment of cold, cough, chronic bronchitis and asthma is effective by leaves of Adhatodavasica. It was also used by traditional midwives at the time of delivery. The leaves of Adhatodavasica are widely used in indigenous remedies. Adhatodavasica include important macro and micro elements: K, Ca, Fe, Cu, Zn and Cr (Manoj et al., 2014). It is also believed to have abortifacient properties. It is used in some parts of India to stimulate uterine contractions, thus speeding childbirth (Atul et al., 2014). Vasica is commonly confirmed to have powerful love potion, soothing, rejuvenate and life dragging out resources (Reddy et al., 2017). The leaves of Adhatodavasica contain many secondary metabolites and phytochemicals. Such as, vasicine, vasicinone, vasicine acetate, 2-acetyl benzyl amine, vasicinolone, vasicol, vasicoline, vasicolinone and adhatodine responsible for its biological properties. It can be used as an eco-safe, biodegradable alternative in prevention and treatment of bacterial infections (Yusuf et al., 2016).

MATERIAL AND METHODS

Collection and Processing of sample: Mature leaves of Adhatodavasica were collected from local area Bhiwandi.

All leaves were washed under tap water separately in batches and shade dried for seven to ten days. Using electric grinder leaves were prepared in powdered and stored in airtight container.

Preparation of leaf extract with various solvents: For each solution, 4gm of leaf powder was added to 40 ml of distilled water, acetone and methanol and stirred constantly for half an hour. The mixture was kept at room temperature for 24 hours. This mixture was kept for boiling, followed by filtration through Whatman filter paper no.1.

ARTICLE INFO

ABSTRACT

The research is about determination of antibacterial activity by aqueous, acetone and methanol extracts of Adhatodavasica and to find the phytochemicals from extract leaves of Adhatodavasica. Herbal have become gradually widespread. Adhatodavasica leaves usually contain two major alkaloids called vasicine and vasicinone which are particular to the Acantheceae family. Leaves and roots of Adhatodavasica contains vasicinosides which are biologically active secondary metabolites. Biological activities like bronchodilator, respiratory stimulant and hypotensive activity are known to possess by vasicinosides. There is an increasing interest in finding antioxidant phytochemicals, because they can prevent the propagation of free radical reactions, that defend human body from diseases. Various phytochemicals are found in Adhatodavasica. The phytochemical components are of organic in nature. Simple compounds are easily converted to complex compounds by bioprocess and used in several therapeutics and medicines.

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India
Test for Phenol
For qualitative determination of phenol 2.0 ml of aqueous, acetone and methanol were taken then 0.5 ml of FeCl₃ solution was added to these extracts where the precipitate of phenol is indicated by formation of intense brown color.

Test for Flavonoid
For qualitative determination of flavonoid 2.0 ml of aqueous, acetone and methanol were taken then 0.5 ml of NaOH solution was added to these extracts where the presence of flavonoid is indicated by formation of intense yellow color that become colorless on addition of few drop of diluted HCl.

Test for Cardiac Glycoside
For qualitative determination of cardiac glycoside 2.0 ml of aqueous, acetone and methanol were taken then 2.0 ml of chloroform solution was added to these extracts then add 2.0 ml of conc. H₂SO₄ solution to these extracts, where the precipitate of cardiac glycoside is indicated by formation of intense layer form and deep brown color.

Test for Triterpene
For qualitative determination of triterpene 2.0 ml of aqueous, acetone and methanol were taken then 0.5 ml of conc. H₂SO₄ solution was added to these extracts, where the precipitate of triterpene was indicated by formation of intense brown ring.

Test for Saponins
For qualitative determination of saponins 2.0 ml of aqueous, acetone and methanol were taken then 20 ml distilled water was added along with extracts separately, the test tube was then shaken in graduated cylinder for 15 minutes, the presence of saponins were indicated by formation of intense 1 cm layer of foam.

Test for Tannins
For qualitative determination of tannins 2.0 ml of aqueous, acetone and methanol were taken then 0.5 ml of conc. H₂SO₄ solution was added to these extracts, then 2.0 ml of Molisch reagent was added, where the precipitate of carbohydrate was indicated by formation of intense violet ring.

Test for Phlobatannin
For qualitative determination of phlobatannin 2.0 ml of aqueous, acetone and methanol were taken then 2.0 ml of 1% HCL solution was added to these extracts, then kept for boiling in water bath, intense red color precipitate indicates the presence of phlobatannin.

Test for Alkaloids
For qualitative determination of alkaloids 2.0 ml of aqueous, acetone and methanol were taken then 2.0 ml Wagner’s reagent solution was added to these extracts, where the precipitate of alkaloids were indicated by formation of intense brownish color.

Test for Steroids
For qualitative determination of triterpene 2.0 ml of aqueous, acetone and methanol were taken then 2.0 ml chloroform solution was added to these extracts, then 2.0 ml of conc. H₂SO₄ solution was added to these extracts, and the precipitate of steroid was indicated by formation of intense yellow with green fluorescence color.

Test for Terpenoids
For qualitative determination of terpenoids 2.0 ml of aqueous, acetone and methanol were taken then 2.0 ml chloroform solution was added to these extracts, and 0.5 ml of conc. H₂SO₄ was added where the precipitate of terpenoid was indicated by formation of intense reddish-brown color.

Test for Reducing Sugar
For qualitative determination of reducing sugar 2.0 ml of aqueous, acetone and methanol were taken then 2.0 ml of Fehling A and Fehling B solution was added to these extracts, and boiled for 5 minutes, the precipitate of reducing sugar indicated by formation of intense orange red color.

Test for Protein
For qualitative determination of protein 2.0 ml of aqueous, acetone and methanol were taken then 2.0 ml of Ninhydrin reagent solution was added to these extracts, and boiled for 5 to 10 minutes in boiling water bath, the precipitate of protein indicated by formation of intense dark purple colour.

In Vitro Determination of Antibacterial Activity of Aqueous, Acetone And Methanol, Plant Extract From Adhatoda Vasica Qualitatively Test: Agar well diffusion method (Indian Pharmacopoeia, 2007)
The colonies of the organism from overnight grown standard cultures of Escherichiacoli ATCC 25922 and Staphylococcusaureae ATCC 6538 were used to make saline suspension. This was further adjusted to 0.5 McFarland’s standard and used as inoculum for assay. Pour plate technique was performed using sterile Mueller Hinton agar. Plates were incubated at 37°C for 24 hours.

RESULTS
Collection of sample: Mature and given leaves of Adhatodavasica were collected from local area Bhiwandi. All leaves were washed under tap water separately in batches and shade dried for seven to ten days. powdered and stored in airtight container and used for further experiment work.
Processing of sample:
Preparation of leaf extract with various solvents: For each solution, 4gm of leaf powder was added to 40 ml of distilled water, acetone and methanol and stirred constantly for half an hour. The mixture was kept at room temperature for 24 hours. This mixture was kept for boiling, followed by filtration through Whatman filter paper no.1

Phytochemical Analysis of Aqueous, Methanol And Acetone Extract
Leaf powder was added to distilled water, methanol and acetone and stirred continuously for half an hour. This solution was kept at room temperature for 24 hours. The solution was then boiled at respective temperature to make hot extract followed by filtration through Whatman filter paper no 1. The result of phytochemical analysis from hot leaves extracted from Adhatodavasica were as shown in table 1.
Table 1 Phytochemicals from extract leaves of Adhatodavasica.

<table>
<thead>
<tr>
<th>Phytochemical Test</th>
<th>Aqueous</th>
<th>Methanol</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac Glycoside</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Protein and Amino acid</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fats</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Reducing Sugar</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: (-) Negative, (+) Positive

Aqueous leaf extract of Adhatodavasica showed presence of all phytochemicals except for cardiac glycoside, phlobatannins and reducing sugar. Methanol leaf extract of Adhatodavasica showed presence of all phytochemicals except for saponins, protein and aminocacid, fat, reducing sugar and phlobatannins. Acetone leaf extract of Adhatodavasica showed presence of all phytochemicals except for carbohydrate, saponins, tannins, carbohydrate, phlobatannins, steroids and reducing sugar. Similar phytochemicals for aqueous leaf extract was obtained by (Thakur et al., 2014, Singh et al., 2014) for acetone leaf extract was obtained by (Yusuf et al., 2016, Keesara et al., 2017) for methanol leaf extract was obtained by (Jayapriya et al., 2015, Thakur et al., 2014, Singh et al., 2014)

*In vitro* Determination of Antibacterial activity of aqueous, Methanol and Acetone plant Extract from adhatoda vasica.

Qualitative study of plant extract of Adhatodavasica done by agar well diffusion method using different dilution of plant extract of Adhatodavasica in the range of 10 mg/ml to 100 mg/ml on standard cultures of Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 6538. The results were as displayed in table 2 and 3 in figure 1 and 2 for plant extract.

Table 2 Determination of Antibacterial Activity by Aqueous, Methanol and Acetone extract of Adhatodavasica leaves

<table>
<thead>
<tr>
<th>Organism</th>
<th>Plant Extract</th>
<th>Concentration of plant extract (mg/ml)</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanol</td>
<td>10 20 30 40 50 60 70 80 90 100</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>Methanol</td>
<td>15 14 16 14 16 14 12 12 14 12 11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>12 12 14 12 12 13 13 12 12 12 17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td>15 13 22 15 14 12 15 17 15 15 16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>15 14 13 13 12 11 11 00 00 00 00</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 6538</td>
<td>Acetone</td>
<td>00 00 00 00 00 00 00 00 00 00 00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td>12 12 16 14 16 11 14 17 15 15 12</td>
<td></td>
</tr>
</tbody>
</table>

The aqueous, acetone and methanol extract leaves of grinder powder of Adhatodavasica showed zone of inhibition against standard cultures of Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 6538 by agar well diffusion method. To compare result of antibacterial activity against test microorganism in the present study literature and reported were available. Hence this kind of comparative study may be first reported and may serve as base line study to evaluated further by researcher in future.

**CONCLUSION**

Adhatodavasica indicated the presence of various phytochemicals in it. Various phytochemicals are present in hot aqueous, acetone and methanol leaf extract of Adhatodavasica. In hot aqueous extract of Adhatodavasica phenol, flavonoids, triterpenes, saponin, tannin, carbohydrates, alkaloids, steroids, terpenoids were present and cardiac glycoside, phlobatannins, protein and amino acid, fat and reducing sugar were absent. In hot methanol extract of Adhatodavasica phenol, flavonoids, triterpenes, saponin, tannin, carbohydrates, alkaloids, steroids, terpenoids were present and cardiac glycoside, phlobatannins, protein and amino acid, fat and reducing sugar were absent. In hot acetone extract of Adhatodavasica phenol, flavonoids, triterpenes, alkaloids, terpenoids, protein and amino acid and fat were present and cardiac glycoside, carbohydrates, tannin, steroids, phlobatannins, saponin were absent. Adhatodavasica indicates the presence of antibacterial activity against Escherichia coli and Staphylococcus aureus. In aqueous extract of Adhatodavasica shows the maximum inhibition against Escherichia coli whereas, Staphylococcus aureus shows the minimum inhibition. In acetone extract of Adhatodavasica shows the maximum inhibition against Escherichia coli whereas, Staphylococcus aureus don’t shows any inhibition. In Methanol extract of Adhatodavasica shows the maximum inhibition against Escherichia coli whereas, Staphylococcus aureus shows the minimum inhibition.

The beneficial activity of powder Adhatodavasica to cure the different types of infections can be determined. Adhatodavasica can be used for the preparation of different types of ointments, face washes, anti-inflammatory lotions, cough-syrup, anti-allergic powder.

**References**

3. Indian Pharmacopoeia. Govt. of India, ministry of health and family welfare India, 2007: 2.2.11-59