PHYTOCHEMICAL INVESTIGATION AND ANTIMICROBIAL ACTIVITY OF MENTHA ARVENSIS L. [PUDINA]

*Patil D. B.¹,², Namrata Palshikar³, Patil S. B.⁴, Chavan S. S.¹, Patil P. J. ⁵ and Bhamburdekar S. B.²

¹Department of Biotechnology, K. B. P. College, Urun –Islampur, Dist- Sangli 415 409, [MS], India.
²P. G. Department of Botany, Plant Physiological Section, Krishna Mahavidyalaya, Rethare Bk. - 415 110, Tal-Karad, Dist-Satara [MS] India.
³Department of Biochemistry, Shivaji University, Kolhapur, [MS], India.
⁴Department of Biochemistry, R. P. Gogate College of Arts & Science and R. V. Jogalekar College of Commerce, Ratnagiri, 415612 [MS] India.
⁵Department of Microbiology, K. B. P. College, Urun - Islampur, Dist-Sangli 415 409 [MS] India.

ABSTRACT

Pudina [Mentha arvensis L.] is a one of the most important aromatic, stimulant medicinal plant belongs to family Lamiaceae. The aim of present investigation is to assess phytochemical and antimicrobial activity of leaves extracts of pudina [Mentha arvensis L.]. In present investigation, in vitro antimicrobial activity studied against pathogenic bacteria such as Staphylococcus aureus, E. coli and Salmonella typhi by agar well diffusion method. The air dried homogenized powder of pudina were subjected in distilled water, acetone and methanol for solvent extraction. The methanol and acetone extract showed maximum zone of inhibition against Staphylococcus aureus, E. coli and Salmonella typhi over the control. The phytochemical study reveals the presence of flavonoids, saponin, alkaloids, tannins, terpenoids and cardiac glycosides in different solvent extracts. Thus, present investigation can be help to discover new bioactive components for the development of new drugs.

KEYWORDS: Mentha arvensis L., Pudina, Phytochemical, antimicrobial activity.
INTRODUCTION
In recent times, the identification and search for compounds with antimicrobial activity has gained more importance because, the rate of infection by antibiotic resistant microorganism increases exponentially. Derrida.\(^1\) remarked that, the increased use of antibiotic may develop antibiotic resistant bacteria. Rajlakshmi \textit{et al.}\(^2\) reported that, Indian medicinal plants are routinely used in medicine because of cost effectiveness and minimal side effects. According to Samy and Ignacimuthus,\(^3\) biological active compounds from natural source have always be a great interest on infectious diseases. It is need to investigate newer phyto-drugs with lesser resistance.

Recently, many developed and developing countries use traditional medicine for primary health care.\(^4\) The uses of aromatic plants in folk medicine are from ancient time. The \textit{Mentha arvensis} L. is a perennial herb which belongs to family Lamiaceae. It is also called as pudina, menthol mint, corn mint. The leaves of pudina are one of the major sources of edible aromatic compounds. The pudina contains highly important volatile oils, resins, tannins, coumarins, alkaloids etc. in large amount. So in the present investigation, we studied phytochemical and antimicrobial screening of leaves of pudina against some pathogenic bacteria.

MATERIAL AND METHOD

Plant Material
Fresh leaves of \textit{Mentha arvensis} L. were collected from botanical garden of K. B. P. College, Urun-Islampur. Dist- Sangli. The collected samples were identified and authenticated in Department of Botany, K. B. P. College, Urun-Islampur, Dist-Sangli. The fresh leaves were washed thoroughly with tap water and followed by double distilled water. Finally, the washed samples were air dried and homogenized to give fine powder. This fine powder was placed in air tight bottle for further study.

Preparation of plant extract
The homogenized powder of mentha leaves were soaked in distilled water, methanol and acetone for solvent extraction at 48 hrs in individual bottles. After solvent extraction, the extracts were filtered through four layered muslin cloth. The final extracts were concentrated by using hot water bath.
Growth and Maintenance of Test Microorganism for Antimicrobial Studies

Antibacterial activity
The three bacterial cultures such as *Staphylococcus aureus*, *E. coli* and *Salmonella typhi* were obtained from Department of Microbiology, K. B. P. College, Urun-Islampur. These bacterial stains were maintained on nutrient agar slant at $37^\circ C$ for further study. The antibacterial activities of leaf extracts were determined by using agar well diffusion method in accordance with Dhairyasheel *et al.*[^5] The antibacterial activity was assessed by measuring of diameter of the zone of inhibition.

Phytochemical screening test-
The phytochemical tests of the different extracts of pudina was conducted in accordance with Njoku and Obi[^6], Abb *et al.*,[^7] and Edego *et al.*[^8]

Test for Flavonoids
In 1 ml extracts of different solvents, add 1 ml 10 % lead acetate solution. The formation of a yellow precipitation indicate positive test for flavonoids.

Test for Saponins
In 2 ml leaves extracts, add small amount of 2 N HCl, shake well and decand the aqueous layer. Finally, add 2 drops of Mayer's reagents. The formation of intense colour foaming lather was taken as positive test for saponin.

Test for Alkaloids
Heat 2 ml leaves extracts of pudina by adding 10 % NaOH solution. The white precipitate was taken as positive test for alkaloids.

Test for Tannins
For tannins, 2 ml leaves extracts of pudina were heated by adding concentrated HNO$_3$ with excess ammonia. The formation of white precipitation indicates the presence of tannins.

Test for Carbohydrate [Molisch’s Test]
The leaves extracts of pudina were treated with Molisch reagent and concentrated sulphuric acid. The reddish violet ring shows the presence of carbohydrate.
Test for Terpenoid
Mixed, 5 ml of leaves extracts with 2 ml of chloroform and then, 3 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown coloration at the interface indicates the presence of the terpenoids.

Test for Cardiac glycosides
0.5 gm leaf extracts diluted by 5 ml distilled water, add 2 ml of glacial acetic acid containing one drop ferric chloride solution. Finally, carefully add 1 ml concentrated sulphuric acid. A brown ring at the interphase indicated the presence of cardiac glycosides.

RESULT AND DISCUSSION
The qualitative phytochemical analysis of leaves of pudina was carried in present study and summarized in table.1. The phytochemical results revealed the presence of various bioactive secondary metabolites in the different solvent extracts of leaves of pudina. The maximum secondary metabolites were observed in acetonic and methanolic extracts. The flavonoid content was more observed in all solvent extracts while, on other hand the carbohydrate content was very scanty in same. The saponin and alkaloids content were observed only in acetonic extract.

The antimicrobial activity of leaf extracts was examined against pathogenic bacteria by measuring zone of inhibition [Table-2]. The results revealed that, the high antimicrobial activity of leaf extracts of pudina were observed in methanolic and acetonic solvents against *Staphylococcus aureus*, *E. coli* and *Salmonella typhi* pathogenic bacteria. The maximum zone of inhibition was recorded in methanolic extracts against *Staphylococcus aureus* over the control. Similar results were reported by Sugandhi and Meera.[9] According to them, antimicrobial activity of the leaf extracts of pudina was more effective against *Staphylococcus aureus* than other studied bacteria.

Table- 1. Phytochemical analysis of leaves extracts of pudina [*Mentha arvensis*]

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Phytochemical analysis</th>
<th>Distilled Water</th>
<th>Acetone</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Terpenoid</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Cardiac Glycosides</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

[+] = Present; [-] = Absent
Table-2. Antimicrobial Activity of pudina [*Mentha arvensis*] on different organism on Nutrient Agar

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Test Microorganism</th>
<th>Diameter of zone of inhibition [mm] [Mean ± SD]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Distilled Water</td>
</tr>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>8±0.22</td>
</tr>
<tr>
<td>2</td>
<td><em>E. coli</em></td>
<td>15±0.55</td>
</tr>
<tr>
<td>3</td>
<td><em>Salmonella typhi</em></td>
<td>8±0.33</td>
</tr>
</tbody>
</table>

Values are mean of three replicate.

CONCLUSION

Thus present study revealed that, Pudina [*Mentha arvensis*] is rich in a wide variety of secondary metabolites and it can be used as therapeutic agent to cure various bacterial infections.

ACKNOWLEDMENTS

The authors would like to thanks Prof. P. V. Gaikwad, Head, Department of Biotechnology, K. B. P. College, Urun- Islampur, Dist- Sangli, Maharashtra, India for providing facilities for research work.

REFERENCES

