ANTI BACTERIAL POTENTIAL OF DIFFERENT EXTRACTS OF *TAGETES ERECTA* LINN

Kiranmai M and Mohammed Ibrahim*

Department of Medicinal Chemistry, Pharmacology and Biotechnology, Nizam Institute of Pharmacy, Deshmukhi, Pochampally (Mandal), Near Ramoji Film City, Nalgonda 508284, Andhra Pradesh & Asian Institute of Advance Research, Hyderabad 500058, Andhra Pradesh, India.

*Corresponding author e-mail: ibrahim-cce@rediffmail.com

ABSTRACT

The present study was carried out to investigate the antibacterial effect of different extracts of leaves and flowers of *Tagetes erecta* Linn. After performing preliminary phytochemical screening and thin layer chromatography, antibacterial study was evaluated according to the agar diffusion method by using gram positive B.cereus, S. aureus and gram negative E.coli, P. aeruginosa. This study was shown that pet ether extract of leaves and ethylacetate extract of flower of *Tagetes erecta* significantly inhibit the growth of bacteria dose dependently.

Keywords: *Tagetes erecta* Linn, Antibacterial activity, Gram positive and Gram negative organism

INTRODUCTION

In the indigenous health care delivery system, numerous plant species and natural products derived from plants are to treat diseases of infectious origin.\(^1\) Medicinal plants represent a rich source from which antimicrobial agents may be obtained. Plants are used medicinally in different countries and are a source of many potent and powerful drugs.\(^2\) Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries.\(^3\) Clinical microbiologists have great interest in screening of medicinal plants for antimicrobial activities and phytochemicals as potential new therapeutics. The active principles of many drugs found in plants are secondary metabolites.\(^4,5\) The beneficial medicinal effects of plant materials typically result from the combinations of secondary metabolites present in the plant such as alkaloids, steroids, tannins and phenolic compounds, flavonoids, resins, fatty acids and gums which are capable of producing definite physiological action on body. The reason for choosing herbs as antibacterial sources is the development of a drug resistance in human pathogens against commonly used antibiotics.\(^6,7\)

The medicinal plant, *Tagetes erecta* Linn. (Family, Asteraceae) widely used in olden days for the treatment of wounds. It is commonly known as aromatic annual herb reaches 0.4-1 m height. It is very popular as a garden plant and yields a strongly aromatic essential oil (tagetes oil), which is mainly used for the compounding of high-grade perfumes. Different parts of this plant including flower are used in folk medicine to cure various diseases.\(^8\) The leaves are reported to be effective against piles, kidney troubles, muscularpain, ulcers, and wounds. The pounded leaves are used as an external application to boils and carbuncles. It is reported to have antioxidant, antymycotic, analgesic activity and 18 active compounds are identified by GC-MS, many of...
them are terpenoids.\textsuperscript{9-11} The flower is useful in fevers, epileptic fits (Ayurveda), astringent, carminative, stomachic, scabies and liver complaints and is also employed in diseases of the eyes. They are said to purify blood and flower juice is given as a remedy for bleeding piles and also used in rheumatism, colds and bronchitis.\textsuperscript{12-13} Phytochemical studies of its different parts have resulted in the isolation of various chemical constituents such as thiophenes, flavonoids, carotenoids and triterpenoids. The plant \textit{T. erecta} has been shown to contain quercetagetin, a glucoside of quercetagetin, phenolics, syringic acid, methyl-3, 5-dihydroxy-4-methoxy benzoate, quercetin, thiethyl and ethyl gallate.\textsuperscript{9,13}

The present study has been undertaken to ascertain the antibacterial potential of leaves and flowers of \textit{Tagetes erecta Linn} and their comparison.

**MATERIALS AND METHODS**

**Plant collection:** Fresh leaves and flowers of \textit{Tagetes erecta} were collected from the adjoining areas of Deshmukhi village, during the month of December to January and taxonomically identified by Professor S. Seetharam rao, Department of Botany, Osmania University, Hyderabad, where a voucher specimen (0333 AUOH) has been deposited.

**Preparation of extracts:** Collected plant material was dried under shade and ground in to coarse powder. Powder so obtained was subjected to soxhlet extraction in order to prepare whole extract and also successive solvent extracts.

**Scheme for preparation of whole extract**

\textbf{coarse powder of leaves / flowers} \hspace{2cm} 70\% ethanol \hspace{2cm} \text{ethanol extract} \hspace{2cm} \text{residue} \hspace{2cm} L_1, F_1

\textbf{L}_1: \text{whole extract of leaves, F}_1: \text{whole extract of flowers}

100g of dried coarse powder of leaves was weighed and packed loosely in thimble of soxhlet placing thin layer of cotton at the bottom and care was taken that the powder doesn’t enter the distillation path. Porcelain chips were placed in the round bottomed flask to avoid bumping of the solvent and the thimble was fit into the mouth of RBF. Ethanol was made to run through the powder for three siphons and soxhlet was equipped with condenser. The solvent was heated to reflux at the boiling point of ethanol that is at the range of 75 -78 °C. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, for 5 hours. The plant material was removed from the thimble and kept for drying. The solvent was recovered and was placed on heating mantle for evaporation to get dry extract. Same procedure was followed to prepare whole extract of flowers also.

**Preparation of successive extracts of leaves and flowers:** Successive extraction was done with the solvents based on their decreasing order of polarity as given below: Petroleum ether>Chloroform>Ethyl acetate>Methanol> Ethanol>Water

100g of dried coarse powder of leaves was weighed and packed loosely in thimble of soxhlet placing thin layer of cotton at the bottom and care was taken that the powder doesn’t enter the distillation path. Porcelain chips were placed in the round bottomed flask to avoid bumping of the solvent and the thimble was fit into the mouth of RBF. Petether was made to run through the powder for three siphons and soxhlet was equipped with condenser. The solvent was heated to reflux at the boiling point of petether that is at the range of 60-80°C. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, for 5 hours. The drug was removed from the thimble and kept for drying. The solvent was recovered and was placed on heating mantle for evaporation to get dry extract. Same procedure was followed to prepare...
other extracts of leaves and flowers by using successive solvents in the above mentioned order.

**Qualitative analysis for different chemical constituents:** All prepared plant extracts were subjected for the qualitative analysis to detect chemical constituents using standard procedures.\(^{15-16}\)

**Thin layer chromatography (TLC)** \(^{17}\): All extracts were subjected to TLC by using falling mobile phase: Ethyl acetate: formic acid: glacial acetic acid: water =100:11:11:20 for the detection of quercetin.

**RESULTS AND DISCUSSION**

The results of the phytochemical screening of leaves and flowers of *Tagetes erecta* were as presented in table 1. These classes (such as alkaloids, glycosides, saponins, tannins, flavonoids and terpenoids) of compounds are known to have curative activity against several pathogens and therefore could suggest the use of traditionally for the treatment of various illnesses.\(^{19-20}\) The antibacterial activities of these extracts could be as a result of the plant secondary metabolites (alkaloids, glycosides, flavonoids, tannins and terpenoids) present in the extracts. Based on earlier reports, flowers of *Tagetes erecta* contains significant amount of free flavonoids and flavonoid glycosides.\(^{21-22}\) Leaves of *Tagetes erecta* contains essential oils including terpenoids and flavonoids.\(^{23-25}\)

Table 2 shows the results of TLC. These results are supporting the above fact of presence of antibacterial constituents in various extracts of *Tagetes erecta*. The results of the antibacterial determination for all the extracts of leaves and flowers of *Tagetes erecta* against the four bacterial species were investigated by agar diffusion method. The agar diffusion method for antibacterial activity showed significant reduction in bacterial growth in terms of zone of inhibition. The zone of inhibition increases on increasing the concentration of extract.

It was found that, all extracts of *Tagetes erecta* were showing antibacterial activity except *L*\(_4\) i.e. ethanolic extract of leaves. *L*\(_2\) (1000mg/ml), *F*\(_2\)(500mg/ml) and *F*\(_4\) (1000mg/ml) were showing the maximum antibacterial activity against Bacillus cereus, *Pseudomonas aeruginosa* and *E.coli* respectively. *L*\(_2\), pet ether extract of leaves was showing maximum activity against both human pathogenic gram positive and gram negative bacteria. The main components of pet ether extract were limonene, β-ocimene, isocamphanone, verbenone, β-caryophylline, tagetone and terpenolene. All these components reported previously to possess antibacterial action against both gram positive and gram negative bacteria.\(^{26}\) Pet ether being a non polar solvent could able to extract terpenoids from leaves.

Next to *L*\(_2\), *L*\(_4\) i.e ethyl acetate extract of leaves was showing promising activity against gram positive bacteria and against *Pseudomonas aeruginosa*. It was not showing any activity against *E.coli*. *L*\(_3\), chloroform extract was not showing activity against
Bacilli cereus and E.coli. and L6, ethanolic extract of leaves were not showing any antibacterial activity. In case of flower extracts, F4 i.e ethyl acetate extract was showing maximum activity when compared to other extracts. The main components of ethyl acetate extract were quercetagetin -7-O-glucoside, all-trans and cis isomers of zeaxanthin, all-trans and cis isomers of lutein, and lutein esters. Quercetagetin -7-O-glucoside is a flavanoid glycoside and reported to have antioxidant activity. Zeaxanthin and lutein have been reported to possess nutritional value and antioxidant property. Even TLC was showing the presence of quercetin in extracts. Next to F4, F2 i.e pet ether extract was showing significant activity against gram negative bacteria and activity against gram positive bacteria. The main components of pet ether extract were limonene, cis caryophylline, mycenol and cadinene and it also contains carotenoids like lutein and zeaxanthene. In comparison with flower extracts leaves extracts were showing promising activity against both gram positive and gram negative bacteria as maximum terpenes and terpene ketones have been present in pet ether extract of leaves.

**CONCLUSION**

Whole extracts and successive extracts of leaves and flowers of *Tagetes erecta* were successfully prepared by using soxhlet extraction procedure and all extracts were subjected to qualitative phytochemical screening for the identification of chemical constituents. All extracts were screened for their antibacterial activity by agar diffusion method and results were evaluated by comparing among leaf extracts and by comparing among flower extracts and also by comparing between leaf and flower extracts. Pet ether extract of leaves and ethyl acetate extract of flowers of *Tagetes erecta* were showed maximum antibacterial activity when compared to other extracts of leaves and flowers. When compared to ethyl acetate extract of flower, pet ether extract of leaves was shown promising activity. Further quantification of chemical constituents in different extracts and detailed study of their biological activity is suggested.

**Table 3: Antibacterial activity of different extracts of *Tagetes erecta***

<table>
<thead>
<tr>
<th>Test samples</th>
<th>Zonal inhibition (mm)</th>
<th>Bacillus cereus Gram+ve</th>
<th>Staphylococcus aureus Gram+ve</th>
<th>E.coli Gram-ve</th>
<th>Pseudomonas aeruginosa Gram-ve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500mg/ml</td>
<td>1000mg/ml</td>
<td>500mg/ml</td>
<td>1000mg/ml</td>
<td>500mg/ml</td>
</tr>
<tr>
<td>L1</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>L2</td>
<td>++</td>
<td>+++*</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>L3</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>L4</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>L5</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>L6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F1</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>F2</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>F3</td>
<td>+++</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F4</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>F5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>F6</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

+: Zone of inhibition (mm) = 6-8, ++: Zone of inhibition (mm) = 8-14, +++: Zone of inhibition (mm) = 14-18
*: significant antibacterial activity
Scheme of successive extraction procedure and their yield in % w/w

coarse powder of leaves / flowers

\[ \begin{align*}
60-80^\circ C & \text{ petether} \\
\text{petether extract} & \rightarrow \text{residue} \\
18.10, 21.81 & \text{below } 60^\circ C \text{ chloroform} \\
\text{chloroform extract} & \rightarrow \text{residue} \\
2.10, 4.65 & 76-78^\circ C \text{ ethyl acetate} \\
\text{ethyl acetate extract} & \rightarrow \text{residue} \\
2.15, 4.87 & 64-66^\circ C \text{ methanol} \\
\text{methanol extract} & \rightarrow \text{residue} \\
2.19, 7.69 & 75-78^\circ C \text{ ethanol} \\
\text{ethanol extract} & \rightarrow \text{residue} \\
2.22, 2.77 &\end{align*} \]

\( L_2, L_3, L_4, L_5 \) and \( L_6 \) are petether, chloroform, ethyl acetate, methanol and ethanol extracts of leaves respectively. \( F_2, F_3, F_4, F_5 \) and \( F_6 \) are petether, chloroform, ethyl acetate, methanol and ethanol extracts of flowers respectively.

Table 1: Qualitative analysis for different chemical constituents of \( Tagetes erecta \)

<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>Whole extract</th>
<th>Petether extract</th>
<th>Chloroform extract</th>
<th>Ethyl acetate extract</th>
<th>Methanol extract</th>
<th>Ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( L_1 )</td>
<td>( F_1 )</td>
<td>( L_2 )</td>
<td>( F_2 )</td>
<td>( L_3 )</td>
<td>( F_3 )</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+</td>
<td>+</td>
<td>++*</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroids&amp;terpenoids</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

++*, present and more significant; +, present and significant; -, absent
Table 2: \( R_f \) values of different extracts

<table>
<thead>
<tr>
<th>Extract</th>
<th>( L_2 )</th>
<th>( L_3 )</th>
<th>( L_4 )</th>
<th>( F_2 )</th>
<th>( F_3 )</th>
<th>( F_4 )</th>
<th>( F_5 )</th>
<th>( F_6 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R_f ) value</td>
<td>0.7</td>
<td>0.9</td>
<td>0.9</td>
<td>0.7</td>
<td>0.8</td>
<td>0.7, 0.8</td>
<td>0.9, 0.7</td>
<td>0.9</td>
</tr>
</tbody>
</table>

REFERENCES