Phytochemical Screening of Aqueous, Acetone, Ethanol and Methanol Leaf Extracts of Ocimum teniflorum, Ocimum gratissimum and Ocimum sanctum

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Abstract
The experiment was carried out during November, 2019 to July, 2020 at Department of Food Safety and Quality Assurance, College of Food Science and Technology, Pulivendula, ANGRAU, Andhra Pradesh, India. A large number of plants show enormous versatility in synthesizing complex materials which have no immediate obvious effect on growth or metabolic functions. Phytochemicals are naturally occurring and biologically active components that have potential disease inhibiting capabilities. Phytochemicals are effective in combating or preventing disease due to their antioxidant effect. The most important of these phytochemicals are alkaloids, tannins, flavonoids, saponins and phenolic compounds. Ocimum is a well-known medicinal plant that consists of various biochemically active components which have many functional effects. The leaf extracts were prepared by using aqueous, acetone, ethanol and methanol solvents and phytochemical analysis was conducted for tannins, saponins, phlabotannins, flavonoids, terpenoids, glycosides and steroids. The results revealed that tannins were found in all the extracts of three Ocimum spp., Saponins were present in all extracts except methanol extract of Ocimum spp., the presence of phlabotannins were observed only in aqueous extract Ocimum spp., Flavonoids were present in aqueous and methanol extracts of Ocimum spp., Glycosides were found in ethanol and methanol extracts of Ocimum spp., The presence of steroids was observed in all extracts except aqueous of Ocimum spp., Terpinoids were present in all extracts except aqueous extract of O. teniflorum and O. gratissimum.

Keywords: Acetone, aqueous, aqueous, ethanol, methanol extract, Ocimum spp.

1. Introduction
The plant kingdom is known to comprise approximately 500,000 plant species that are found worldwide, of which only 1.0% has been phytochemically investigated with an illimitable potential for discovering novel bioactive compounds mainly in medicinal plants (Rodrigues et al., 2007). The medicinal plants are rich in secondary metabolites [which are potential sources of drugs] and essential oils of therapeutic importance. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils (Janssen et al., 1987), as well as in tannin (Saxena et al., 1994). The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability (Siddiqui, 1993). The plants should be investigated to better understand their properties, safety and efficiency (Eloff, 1998). Plants show enormous versatility in synthesizing complex materials which have no immediate obvious growth or metabolic functions. Phytochemicals are effective in combating or preventing disease due to their antioxidant effect. The medicinal and antimicrobial properties of plants lie in their component phytochemicals (Akinmoladun et al., 2007).

The World Health Organization country relies on traditional medicines, mostly plant drugs, for their primary health care needs. Further, a large number of phyto-drugs are popular and or rather harmless effects (Shahavi and Desai, 2008). Ethnobotanical literature of India, several hundreds of plants are known to have the potential to treat many diseases and one of those popular ones is Tulasi traditionally used for the treatment of diseases (Das et al., 2009). Leaves possess antimicrobial activity. Infections with both Gram-positive and Gram-negative bacteria have clinically become intractable, slowly, due to the emergence of multidrug resistant (Naik et al., 2015).

Ocimum spp. is one such medicinal plant that is grassy and annual whose leaves are oval with a sharp tip. It is a native of Iran, Afghanistan and India (Mann et al., 2000). This species belongs to the mint family, Lamiaceae spp., commonly known as “holy basil” in English and “Tulasi” in Hindi and Sanskrit. Morphologically, there are many types of tulasi, some of them are a purplecolored leaf or dark variety, commonly known as “krishna tulasi” (Ocimum teniflorum), a green colored leaf or light variety known as “rama tulasi” (ocimum sanctum) and...
other type is “vana tulasi” (Ocimum gratissimum) (Kothari et al., 2005). Its oil possesses a pleasant odor characteristic of the plant, with an appreciable note of clove. The chemical composition of the oil of O. tenuiflorum has been the subject of previous studies (Lawrence et al., 1972, Lawrence et al., 1980, Dey and Choudhuri, 1983, Malik et al., 1986, Laakso et al., 1990, Pino et al., 1998., Machado et al., 1999, Maheshwari et al., 1987). In last few decades several studies have been carried out by Indian scientists and researchers to suggest the role of essential oils &eugenol in therapeutic potentials of Ocimum sanctum L. (Sen, 1993). Eugenol is a phenolic compound and major constituent of essential oils extracted from different parts of Tulsi plant (Gupta et al., 2002, Khanna and Bhatia., 2003).

The blessed Basil or Tulsi is significant in the traditional Ayurvedic and Unani systems (Pattanayak et al., 2010). In India, Ocimum sanctum is believed that it can be given the treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, painful eye diseases, increase in body temperature and also insect bite (Xia et al., 2018). Different parts of Tulsi plant e.g. leaves, flowers, stem, root, seeds etc. are known to possess therapeutic potentials and have been used, by traditional medical practitioners, as expectorant, analgesic, anticancer, antiasthmatic, antiemic, diaphoretic, antiabetic, antifertility, hepatoprotective, hypotensive, hypolipidmic and antistress agents (Prakash and Gupta, 2005). Keeping in views, the study was aimed to find phytochemical compounds responsible for the antimicrobial activities.

2. Materials and Methods

2.1. Collection and preparation of plant material
Tulsi spp., such as Ocimum sanctum, Osmium teniflorum, Ocimum gratissimum were collected from Professor Jayashankar Telangana State Agricultural University, Hyderabad, Telengana, India from November, 2019 to July, 2020. The fresh leaves collected were washed in clean water followed by distilled water. The cleaned leaves were shade dried at room temperature and powdered. The powdered materials were stored in air-tight jars at room temperature.

Soak 20 g of Tulasi powder in 200 ml of solvent for 48 h. Decant the solvent again soak the residue with the same solvent for 24 h. Combine the total extract. Filter the solution by Whatman’s filter paper No.1. Evaporate the solution in a rotary evaporator at 40°C. Add respective solvent to make up for the required volume.

2.2. Phytochemical Screening
Chemical tests were carried out by using the aqueous and organic solvent extracts of Tulasi to identify the presence of phytochemical constituents by using the standard procedure described by Harborne (1998).

2.3. Test for Tannins
Take 2.0 ml of aqueous extract. Stir after adding 2.0 ml of distilled water. Add a few drops of FeCl₃ solution. The formation of green color precipitate indicates the presence of tannins

2.4. Test for Saponins
Take 5.0 ml of aqueous extract. Shake it vigorously with 5.0 ml of distilled water and warm. The formation of stable foam indicates the presence of saponins.

2.5. Test for phlobatannins
Take 2.0 ml of 1% HCl and add 2.0 ml of aqueous extract. Boil the mixture. Deposition of a red precipitate indicates the presence of phlobatannins.

2.6. Test for Flavonoids
Take 1.0 ml of aqueous extract and add 1.0 ml of 1.0% lead acetate solution. The formation of yellow color indicates the presence of flavonoids.

2.7. Test for Terpenoids
Take 2.0 ml of organic extract and dissolve it in 2.0 ml of chloroform. Evaporate the mixture to dryness and add 2.0 ml of H₂SO₄ and heat for about 2.0 m. The development of greyish color indicates the presence of terpenoids.

2.8. Test for Glycosides
Take 2.0 ml of organic extract and dissolve it in 2.0 ml of chloroform. Add 2.0 ml of acetic acid to the mixture. Cool this solution with ice and add H₂SO₄ carefully. Color change from violet to blue-green indicates the presence of glycosides.

2.9. Test for Steroids
Take 2.0 ml of organic extract and dissolve it in 2.0 ml of chloroform. Add 2.0 ml concentrate H₂SO₄ to the solution. The formation of red color in the lower chloroform layer indicates the presence of steroids (or) take 2.0 ml of organic extract. Dissolve it in 2.0 ml of chloroform. Treat the solution with H₂SO₄ and acetic acid. The development of greenish color indicates the presence of steroids.

3. Results and Discussion

3.1. Phytoconstituent screening
hydrate, Elemol, Tetradecanal, Selin-11-en-4-α-ol, 14-hydroxy-
α-humulene. Alcoholic extract of leaves/aerial parts contain
Urosolic acid, Apigenin, Luteolin, Apigenin-7-Oglucuronide,
Luteolin-7-O-glucuronide, Isorrientin, Orientin, Molludistin,
Stigmasterol, Triacantanol ferulate, Vicenin-2;Vitexin,
Isovitexin, Aesculetin, Aesculin, Chlorgenic acid, Galuteolin,
Circineol, Gallic acid, gallic acid methyl ester, Procatechuic acid,
Vallinin acid, 4-hydroxybenzoic acid, Caffeic acid, Chlorogenic
acid, Phenylpropane glucosides, β-Stigmasterol, urosolic
acid. Fixed Oil from Seeds contain Palmitric acid, Stearic
acid, Linolenic acid, Oleic acid, Sitosterol, Dilinoleno-linolins,
Linodilinolin, Hexourenic acid. Mineral Content 100 g
are
vitamin C (83 μg), Carotene (2.5 μg), Ca (3.15%), P (0.34%)
, Cu (0.4 μg), Zn (0.15 μg), V (0.54 μg), Fe (2.32 μg),
Ni (0.73 μg) (Mondal et al., 2007).

In the present study, the Phytoconstituent screening
showed the presence of Tannins, Saponins, Phlabotannins,
Flavonoids, Terpinoids, Glycosides and Steroids. From Table
1 results revealed that tannins were found in all the extracts
except methanol extract of Ocimum spp., the presence of
phlabotannins were observed only in aqueous extract Ocimum
spp., Flavonoids were present in aqueous and methanol
extracts of Ocimum spp., Glycosides were found in ethanol
and methanol extracts of Ocimum spp., The presence of
steroids was observed in all extracts except aqueous Ocimum
spp., Terpenoids were present in all extracts except aqueous
extract of O. teniflorum and O. gratissimum.

Table 1: Phytochemical properties of the leaf extracts of Ocimum spp.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Treatment</th>
<th>Ocimum spp.</th>
<th>Tannin</th>
<th>Saponnin</th>
<th>Phlabotannin</th>
<th>Flavonoid</th>
<th>Terpinoid</th>
<th>Glycoside</th>
<th>Steroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Aqueous</td>
<td>O. teniflorum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O. gratissimum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O. sanctum</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Acetone</td>
<td>O. teniflorum</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>O. gratissimum</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>O. sanctum</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>3.</td>
<td>Ethanol</td>
<td>O. teniflorum</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td></td>
<td></td>
<td>O. gratissimum</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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<td></td>
<td>O. sanctum</td>
<td>+</td>
<td>+</td>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Methanol</td>
<td>O. teniflorum</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O. gratissimum</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>O. sanctum</td>
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<td>+</td>
</tr>
</tbody>
</table>

Key: + Present, - Absent

Borah and Biswas (2018) results found that various bioactive
molecules were in Tulsi leaf extract from the phytochemical
screening. The amount of extraction is more in the case of
organic solvent than that of water. From the quantitative
analysis, it was found that a high amount of phenols is
present in Tulsi leaf ranging from 1.6 to 7.6%. Consequently,
the amount of alkaloids and flavonoids ranged from 0.91 to
1.28% and 1.56 to 2.24%, respectively. The phytochemical
constituents such as alkaloids, steroids, flavonoids, tannins,
phenols and several other aromatic compounds of plants
serve a defense mechanism against predation by many
microorganisms, insects and other herbivore (Bonjar et al.,
2004). Singh et al. (2013) on phytochemical investigation
discovered the presence of steroidal compounds (appearance
of blue or green color or a mixture of the two shades); alkaloids
and tannins (the turbidity or yellow precipitation shows the
presence of alkaloids and greenish precipitate indicated the
presence of tannins) and absence of flavonoids (not observed
yellow coloration) in all mentioned extracts of the plant. The
phytochemical characteristics of the leaf extract of Ocimum
teniflorum were investigated. The results reveal
the presence of medicinally active constituents like tannins,
alkaloids, terpenoids, steroids and Flavnois, Phlobatannins,
Glycosides in the leaves of ocimum tenuiflorum. While saponins
were absent in these plants (Naik et al., 2015). The present
study provides evidence that leaf extracts of Ocimum spp.,
contain medicinally important bioactive compounds and
their effect on selected bacteria. This justifies the use of plant
species as a traditional medicine for various diseases and the
need to consider them.
4. Conclusion

The identified phytochemical compounds could be the bioactive constituents responsible for the efficacy of the leaves of the studied plants. Thus, the plant extracts could be a source for industrial production of drugs useful for chemotherapy of some microbial infections.

5. Reference


Mann, C.M., Cox, S.D., Markham, J.L., 2000. The outer membrane of Pseudomonas aeruginosa NCTC 6749 contributes to its tolerance to the essential oil of Melaleuca alternifolia (tea tree oil). Letters in Applied Microbiology 30(4), 294–297.


