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A COMPARITIVE STUDY OF THE PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF OCIMUM SANCTUM L AND OCIMUM GRATISSIMUM L.

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Abstract

The present study was conducted to have an awareness regarding the different phytochemicals present in medicinally useful plant species of Ocimum .The study also aimed to find out whether these phytochemicals present may aid in combating most common disease causing microbes. Leaves and stem of O,sanctum and O,gratissimum were separately collected for the study. Qualitative analysis was done with the stem and leaf extracts. Secondary metabolites like alkaloids, terpenoids, phytosterols, saponins, flavonoids, proteins, carbohydrates were present. However Tannins were completely absent in any of these extracts. Antibacterial activity revealed that the plant species has ability to resist diseases. Acetonic extracts were proven to be having more microbicidal effects. O.gratissimum leaves showed more effective control against various microbes selected for the study. O.sanctum stem and O.gratissimum stem showed almost similar microbicidal qualities, but effective against different microbes selected for the study.

Keywords: *Medicinal Plants, Phytochemicals, Antibacterial, Ocimum, species.*

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Introduction

The potential of plants in controlling several dreadful diseases is highly appreciated by people all over the world. India with its vast biodiversity can offer a great role in this regard. India is a country which has a particular system of medicine practised several thousands of years ago- "The Ayurveda "which infact is based on plants and its secondary metabolites. Plants have several secondary metabolites which are of immense potential in the field of medicine. Some of the most important bioactive phytochemical constituents include alkaloids, flavonoids, terpenoids, phenolics, essential oils, tannins , saponins etc.

The phytoconstituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds and so on. The beneficial medicinal effects of plant material typically results from the combinations of secondary metabolites present in the plant. The screening of plants for their potential uses can be done only with the knowledge of the different phytochemicals present in them. Exploration of the chemical constituents of the plants and pharmacological screening will thus provide us the basis for developing new life-saving drugs.

Ocimum sanctum commonly known as Thulasi in Malayalam, Thulsi in Hindi and Holy Basil in English and *ocimum gratissimum* - a popular herb was used for this study.

Ocimum sanctum--The plant is commonly cultivated in gardens and also grown near temples. The medicinal properties attributed to the plant are not only mentioned in Ayurveda, but in Unani, Greek, Siddha and other well known systems of Medicine. *Ocimum Sanctum* contains Vitamin C and A, and minerals like Calcium, Zinc and Iron, as well as chlorophyll and many other phytonutrients. It enhances efficient digestion, absorption and use of nutrients from food and other herbs.

Baby and Vrundha (2003) reported Ethnopharmacological and phytochemical aspects of *Ocimum Sanctum* Linn-The Elixis of life. The plant is a rich source of various components including Eugenol, Oleic acid, Rosmarinic acid, Ocimarin, Isorientin, Isovitexin, Galuteolin, Gallic acid, Vitamin C, Calcium, Phosphorous and many more.

Singh *et al.,* 2005 reported that the Anti-bacterial activity of *Ocimum Sanctum* L. Fixed Oil. *Ocimum Sanctum* fixed oil showed good anti-bacterial activity against Staphylococcus aureus, Bacillus pumilus and *Pseudomonas aeruginosa*, where *Staphylococcus aureus* was the most sensitive organism.

Ocimum gratissimum - is commonly called 'alfavaca' .Phytochemical screening of this plant has revealed the presence of many active ingredients., such as flavonoids, triterpenes, alkaloids, citral, saponins, eugenol, linaol, methyl cinnamate, camphor and thymol. Eugenol, an isolate from *Ocimum gratissimum* has been observed to possess antihelminthic, nematicidal and insecticidal properties. According to the literal use, the oils produced from *Ocimum gratissimum* are active against several bacteria and fungi. Nakamura *et al.,*1999 reported that the antibacterial activity of

Ocimum gratissimum L. essential oil. The essential oil of (EO) *Ocimum gratissimum* inhibited *Staphylococcus aureus*, *Shigella flexineri, Salmonella enteritidis, E. coli, Klebsiella sp and Proteus mirabilis.* Emeka and Elizabeth (2009) reported that justification for the use of *Ocimum gratissimum L* in herbal medicine and its interaction with disc antibiotics.

As time passes, several microbes are gaining resistance against many of the drugs used and consistent screening and evaluvation should be done in combating various modified microbes. A thorough knowledge of the different antimicrobial compounds in plants should be brought to light with the phytochemical extraction , and quantification of the present compounds and an understanding of the roles of these individual compounds is inevitable.

Methodology

The present study was undertaken to analyse the phytochemical and antibacterial properties of two species of the Genus Ocimum which comes under the family Lamiaceae.

1.Collection of materials

The materials for study were collected from Aluva, Varapuzha and from Maharaja's college campus. The plants were identified using flora. The plants taken for the present study were *Ocimum sanctum* and *Ocimum gratissimum.*

2.Drying Procedure

The collected plant materials were washed in tap water for removing dust and dirt. Then they were kept in shade for drying and grind the plant . Then the powder is stored in airtight containers for further studies.

3.Preparation of extract from the plant

20 grams of plant powder were weighed accurately and separately dissolved in distilled water, acetone and petroleum ether and it is centrifuged. The supernatant is collected and then kept it in refrigerator and used for further studies.

4.Preparation of culture medium

1. Nutrient broth medium 2.Nutrient agar medium

5.Phytochemical Analysis

The Acetone, Petroleum ether and Aqueous extracts of *Ocimum sanctum* and *Ocimum gratissimum* stem and leaf parts were used for phytochemical studies as per the standard procedure of phytochemical methods by Herborne J B (1973).

6. Bacterial cultures used in Antibacterial activity.

Five strains of bacteria were used for the study. The strains used for the study are *E.coli, Proteus vulgaris, Klebsiella pneumonia, Streptococcus aureus and Morganella.* The bacterial inoculums were collected from Regional Laboratory Center, Ernakulam.

7.Antimicrobial assay

The experiment was done in laminar air flow chamber. The bacterial culture in nutrient broth was swabbed using buds over the solidified agar medium, then the sterilized filter paper disc were dipped in different extracts of plant parts of both the plants *(ocimum sanctum and ocimum gratissimum)* under study. The filter paper disc is transferred to agar plate. Then the petriplates were incubated for 24 hours . After the stipulated period of time, take out the petriplates and the zone of inhibition was observed and measurements were taken using scale.

Observation and Result

Phytochemical Analysis

The phytochemical analysis of aqueous extract of the two plants studied showed almost similar results. Leaves of ocimum sanctum and ocimum gratissimum showed the presence of alkaloids, terpenoids, proteins, saponins, flavonoids, anthocyanin, phenols, carbohydrates and phytoserols. The stem extracts of the two plants were almost similar. Both showed the presence of Terpenoids, proteins, phytosterols, Flavonoids, phenol, carbohydrates and anthocyanin. However, *O.gratissimum* stem showed the presence of alkaloids which was absent in *O.sanctum* stem. Tannins were altogether absent in both stem and leaf extracts of both the plants studied. Aqueous extracts yield better results than acetone . Petroleum ether extracts gives positive response to the presence of Terpenoids only in case of *O.sanctum amd O.gratissimum* leaf . The stem extracts in petroleum ether bring out the presence of Terpenoids and alkaloids only in case of *O.gratissimum* while in *O. sanctum* no significant result is obtained.

The acetone extract of leaves of two plants shows similar results. It contain alkaloid, terpenoid, phytosterol and phenol but does not contain other chemical compounds. The acetone extract of ocimum sanctum stem showed the presence of terpenoid, protein, phytosterol, flavanoid, phenol and anthocyanin while *Ocimum gratissimum* contains alkaloid, terpenoid, protein and phenol.

Antibacterial activity

Analysis of antibacterial activity of *Ocimum sanctum* and *Ocimum gratissimum* against 5 strains of bacteria *(E.coli, proteus vulgaris, klebsiella pneumoniae, morganella, streptococcus aureus)* was done.

Antibacterial activity of *Ocimum sanctum* leaf against *E.coli* shows that the extraction in water showed an inhibition zone of 6mm in diameter, in acetone it is

8mm and in petroleum ether it is 6mm. Antibacterial activity of *Ocimum sanctum* leaf against *proteus vulgaris* shows that the extraction in aqueous showed inhibition zone of 8mm in diameter, in acetone it is 11mm and in petroleum ether no zone of inhibition is formed. Antibacterial activity of *Ocimum sanctum* leaf against *klebsiella pneumonia* shows that the extraction in aqueous showed inhibition zone of 9mm, in acetone it is 8mm and in petroleum ether it is 5mm. Antibacterial activity of *Ocimum sanctum* leaf against *morganella* shows the result that the extraction in aqueous showed inhibition zone of 8mm , in acetone it is 9mm and in petroleum ether it is 5mm. Antibacterial activity of *Ocimum sanctum* leaf against *streptococcus* aureus shows the result that the extraction in aqueous showed an inhibition zone of 8mm , in acetone it is 10mm and in petroleum ether it is 4mm.

Antibacterial activity of *Ocimum sanctum* stem against *E.coli* shows that the extraction in aqueous showed inhibition zone of 5mm , in acetone it is 10mm and in petroleum ether no clear zone is formed. Antibacterial activity of *Ocimum sanctum* stem against *Proteus vulgaris* shows that the extraction in aqueous showed inhibition zone of 9mm , in acetone it is 8mm and in petroleum ether no clear zone is formed. Antibacterial activity of *Ocimum sanctum* stem against *Klebsiella* pneumonia shows the result that the extraction in aqueous showed inhibition zone of 9mm in diameter, in acetone it is 9mm and in petroleum ether no zone of inhibition is formed. Antibacterial activity of *Ocimum sanctum* stem against *Morganella* shows the result that the extraction in aqueous showed inhibition zone of 8mm in diameter, in acetone it is 9mm and in petroleum ether no inhibitory zone is formed. Antibacterial activity of *Ocimum sanctum* stem against *Streptococcus aureus* shows the result that the extraction in aqueous showed inhibition zone of 6mm , in acetone it is 14mm and in petroleum ether no inhibition zone is formed.

Antibacterial activity of *Ocimum gratissimum* leaf against *E.coli* shows the result that the extraction in aqueous showed inhibition zone of 11mm in diameter, in acetone it is 12mm and in petroleum ether it is 3mm. Antibacterial activity of *Ocimum gratissimum* leaf against proteus vulgaris shows the result that the extraction in aqueous showed inhibition zone of 10mm in diameter, in acetone it is 9mm and in petroleum ether it is 6mm. Antibacterial activity of *Ocimum gratissimum* leaf against *Klebsiella pneumonia* shows the result that the extraction in aqueous showed inhibition zone of 8mm in diameter, in acetone it is 10mm and in petroleum ether no clear zone is formed. Antibacterial activity of *Ocimum gratissimum* leaf against morganella shows the result that the extraction in aqueous showed inhibition zone of 9mm in diameter, in acetone it is 12mm and in petroleum ether no clear zone is formed. Antibacterial activity of *Ocimum gratissimum* leaf against *Streptococcus* aureus shows the result that the extraction in aqueous showed inhibition zone of 9mm in diameter, in acetone it is 10mm and in petroleum ether it is 4mm.

Antibacterial activity of *Ocimum gratissimum* stem against E.coli shows the result that the extraction in aqueous showed inhibition zone of 11mm in diameter, in acetone it is 8mm and in petroleum ether no zone of inhibition is formed. Antibacterial activity of *Ocimum gratissimum* stem against *Proteus vulgaris* shows the result that the extraction in aqueous showed inhibition zone of 9mm in diameter, in acetone it is 8mm and in petroleum ether no inhibition zone is formed. Antibacterial activity of *Ocimum gratissimum* stem against *Klebsiella pneumonia* shows the result that the extraction in aqueous showed inhibition zone of 9mm in diameter, in acetone it is 14mm and in petroleum ether no zone of inhibition is formed. Antibacterial activity of *Ocimum gratissimum* stem against *Morganella* shows the result that the extraction in aqueous showed inhibition zone of 10mm in diameter, in acetone it is 11mm and in petroleum ether no inhibition zone is formed. Antibacterial activity of *Ocimum gratissimum* stem against *Streptococcus aureus* shows the result that the extraction in aqueous showed inhibition zone of 9mm in diameter, in acetone it is 8mm and in petroleum ether no zone of inhibition is formed.

Table 1: Phytochemical Analysis of *Ocimum sanctum leaf*

Phytoconstituents	Test/Reagent	In Distilled water	In Acetone	In petroleum ether
Alkaloids	Mayer's test	+	+	_
	Hager's test	+	+	_
Terpenoid	Copper acetate test	+	+	+
Proteins	Xanthoprotein test	+	_	_
Phytosterols	Libermann Burchard's test	+	+	+
Saponins	Froth test	+	_	_
Flavonoids	Alkaline reagent test	+	_	_
	Lead acetate test	+	_	_
Phenol	Lead acetate test	+	+	_
Carbohydrate	Benedict's test	+	_	_
Tannins	Fecl test	_	_	_
	Lead acetate test	_	_	_
Anthocyanin	Test with con.sulphuricacid	+	_	_

Table 2: Phytochemical Analysis of *Ocimum sanctum Stem*

Phytoconstituents	Test/Reagent	In Distilled water	In Acetone	In petroleum ether
Alkaloids	Mayer's test	–	–	–
	Hager's test	–	–	–
Terpenoid	Copper acetate test	+	+	–
Proteins	Xanthoprotein test	+	+	–
Phytosterols	Libermann Burchard's test	+	+	–
Saponins	Froth test	–	–	–
Flavonoids	Alkaline reagent test	–	+	–
Phenol	Lead acetate test	+	+	–
Carbohydrate	Benedict's test	+	–	–
Tannins	Fecl test	–	–	–
	Lead acetate test	–	–	–
Anthocyanin	Test with con.sulphuricacid	–	+	–

Table 3: Phytochemical Analysis of *Ocimum gratissimum leaf*

Phytoconstituents	**Test/Reagent**	**In Distilled water**	**In Acetone**	**In petroleum ether**
Alkaloids	Mayer's test	+	+	–
	Hager's test	+	+	–
Terpenoid	Copper acetate test	+	+	+
Proteins	Xanthoprotein test	+	–	–
Phytosterols	Libermann Burchard's test	+	+	–
Saponins	Froth test	+	–	–
Flavonoids	Alkaline reagent test	+	–	–
	Lead acetate test	+	–	–
Phenol	Lead acetate test	+	+	–
Carbohydrate	Benedict's test	+	–	–
Tannins	Fecl test	–	–	–
	Lead acetate test	–	–	–
Anthocyanin	Test with con.sulphuricacid	+	–	–

Table 4: Phytochemical Analysis of *Ocimum gratissimum Stem*

Phytoconstituents	Test/Reagent	In Distilled water	In Acetone	In petroleum ether
Alkaloids	Mayer's test	+	+	+
	Hager's test	+	+	+
Terpenoid	Copper acetate test	_	+	+
Proteins	Xanthoprotein test	+	+	_
Phytosterols	Libermann Burchard's test	+	_	_
Saponins	Froth test	_	_	_
Flavonoids	Alkaline reagent test	+	_	_
	Lead acetate test	+	_	_
Phenol	Lead acetate test	+	+	_
Carbohydrate	Benedict's test	+	_	_
Tannins	Fecl test	_	_	_
	Lead acetate test	_	_	_
Anthocyanin	Test with con.sulphuricacid	+	_	_

FIGURE 1

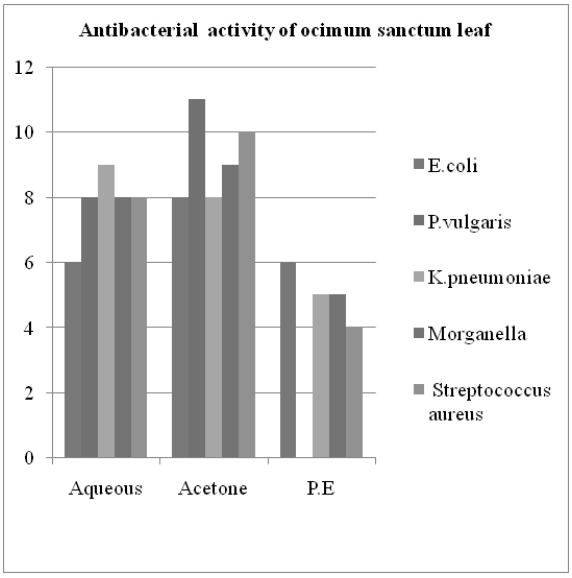


FIGURE 2

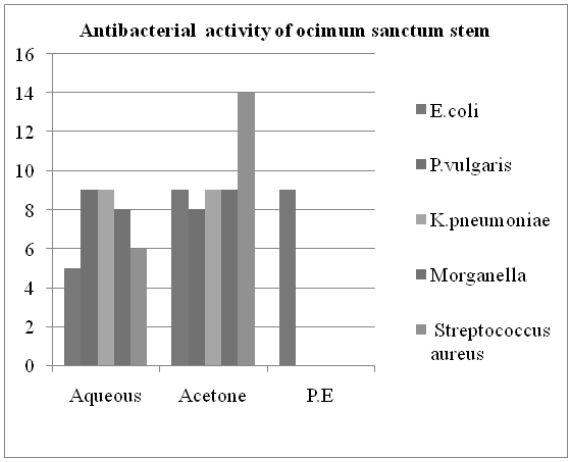


FIGURE 3

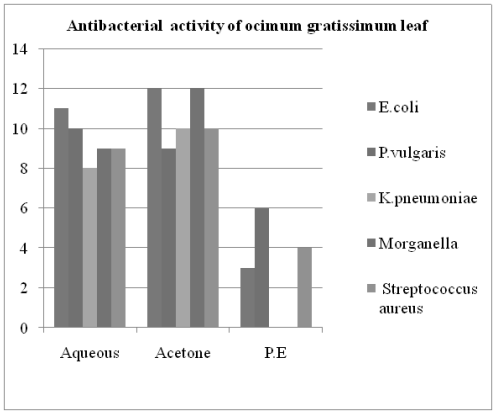


FIGURE 4

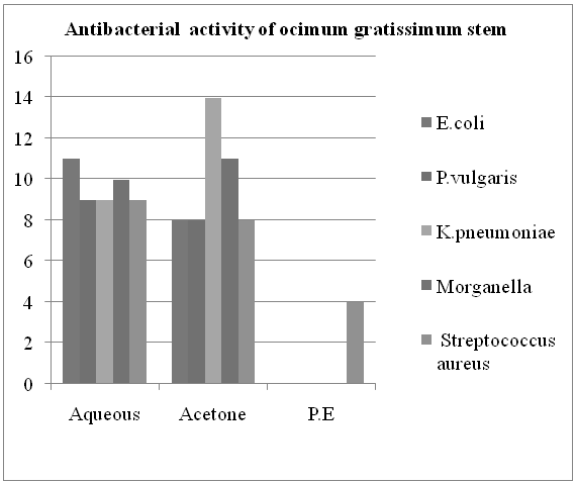


PLATE 1

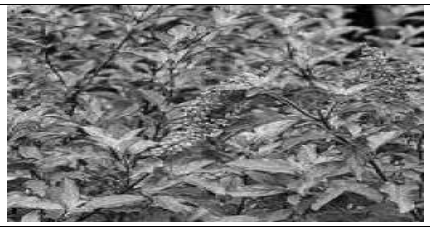
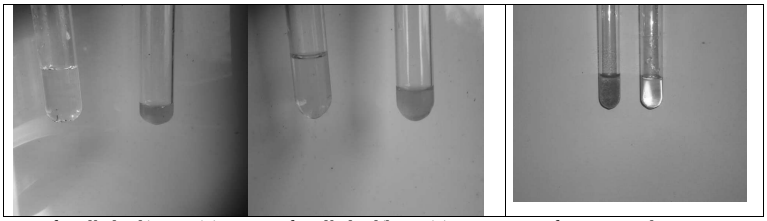
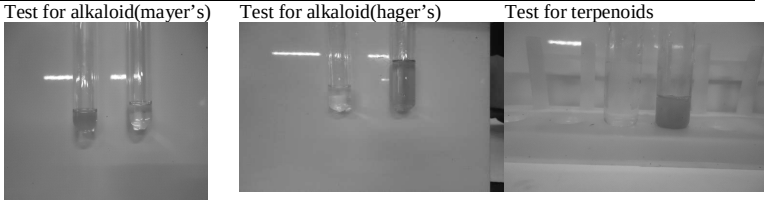
	
Ocimum sanctum L.	***Ocimum gratissimum L.***

PLATE 2

PHYTOCHEMICAL ANALYSIS OF OCIMUM SANCTUM LEAF



Test for alkaloid(mayer's) Test for alkaloid(hager's) Test for terpenoids



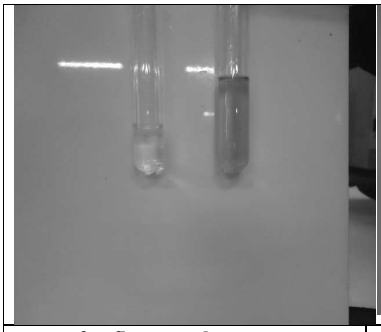
Test for protein Test for flavonoid Test for phenol

Test for carbohydrate Test for phytosterol Test for saponin

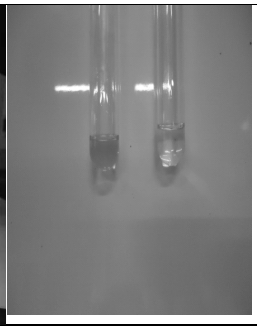
Test for anthocyanin

PLATE 3

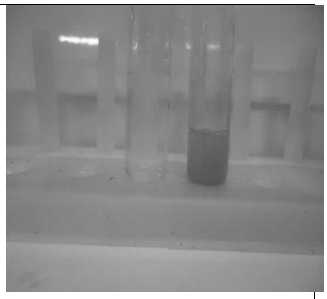
PHYTOCHEMICAL ANALYSIS OF OCIMUM SANCTUM STEM



Test for flavonoid

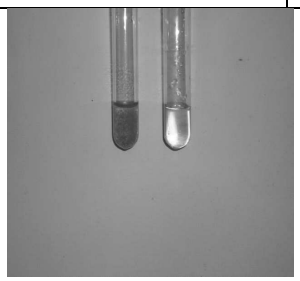


Test for protein

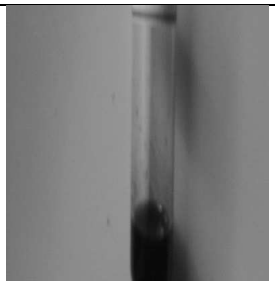


Test for phenol

Test for anthocyanin



Test for terpenoid

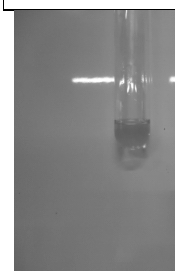


Test for carbohydrate

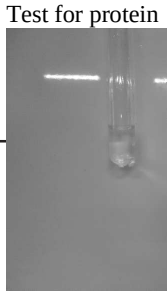
PLATE 4

PHYTOCHEMICAL ANALYSIS OF OCIMUM GRATISSIMUM LEAF

Test for alkaloid



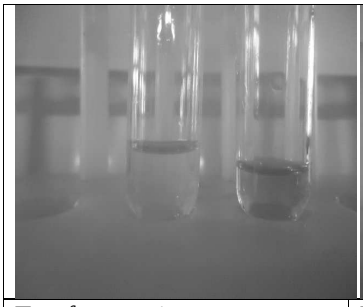
Test for protein



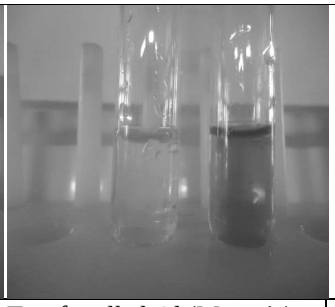
Test for flavanoi

PLATE 5

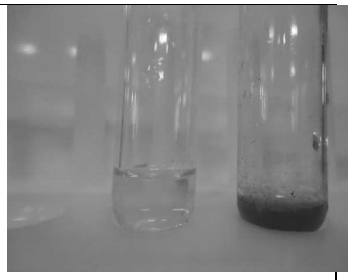
PHYTOCHEMICAL ANALYSIS OF OCIMUM GRATISSIMUM STEM



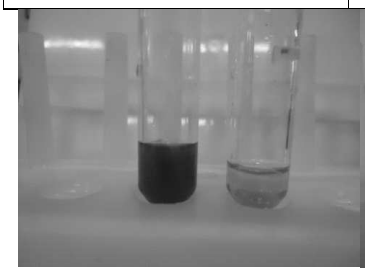
Test for protein



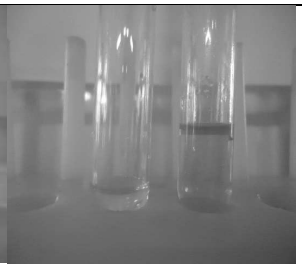
Test for alkaloid (Mayer's)



Test for alkaloid(Hager's)

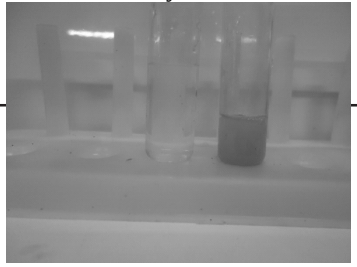


Test for anthocyanin

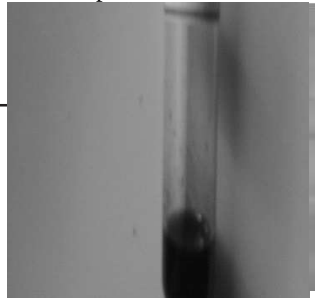


Test for terpenoid

Test for flavonoid



Test for phenol



Test for carbohydrate

Test for phytosterol

PLATE 6

Antibacterial effect of ocimum sanctum and *ocimum gratissimum* against E.coli

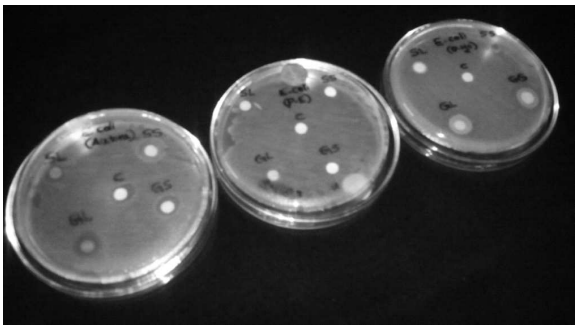


PLATE 7

Antibacterial effect of ocimum sanctum and *ocimum gratissimum* against *proteus vulgaris*

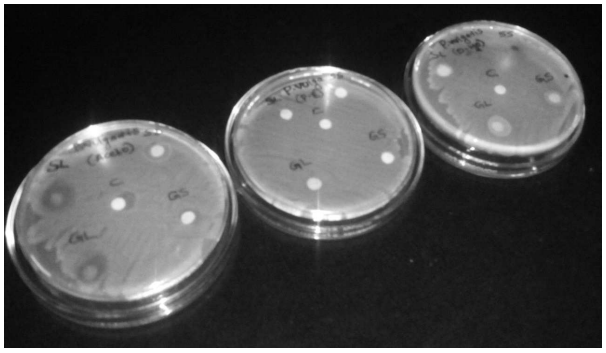


PLATE 8

Antibacterial effect of *ocimum sanctum* and *ocimum gratissimum* against *klebsiella pneumoniae*

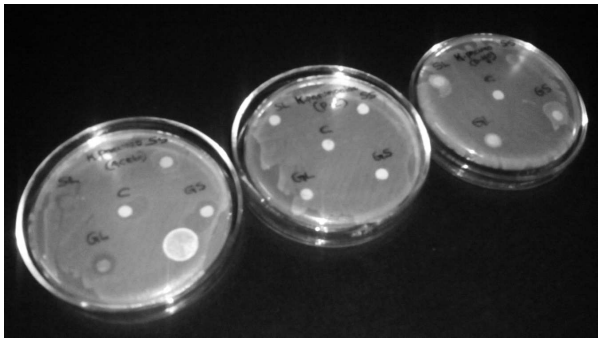


PLATE 9

Antibacterial effect of *ocimum sanctum* and *ocimum gratissimum* against *morganella*

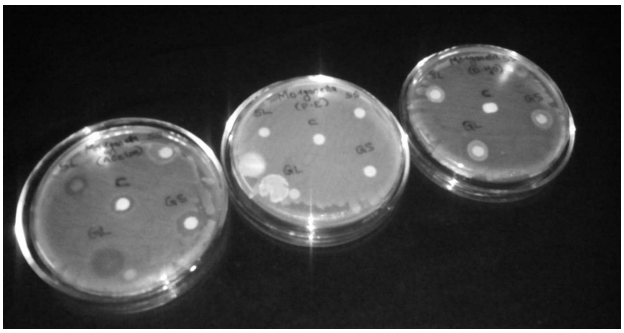
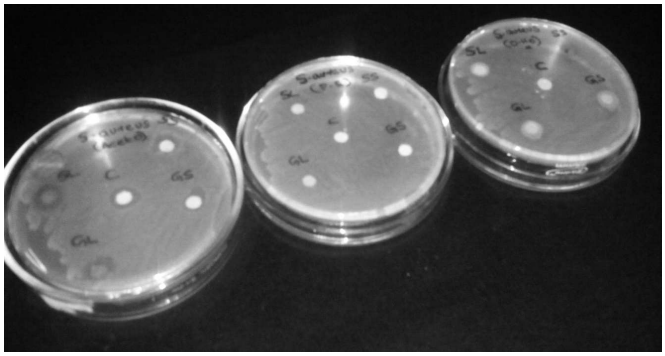


PLATE 10

Antibacterial effect of *ocimum sanctum* and *ocimum gratissimum* against *steptococcus aureus*



DISCUSSION

The present study shows the presence of phytochemical constituents such as alkaloids, saponins, flavonoids, terpenoids, proteins, anthocyanins, carbohydrates and phytosterols in aqueous and Acetonic extracts. Qualitative phytochemical screening and GC-MS analysis of *Ocimum Sanctum* L. Leaves by G Devendran and U Subramanian (2011) showed the presence of phytochemical constituents such as , saponins, flavonoids, steroids, terpenoids and cardiac glycerides. Our study was also in confirmation with this study.

The phytochemical analysis shows that the extraction in aqueous medium brings to light the presence of more chemical compounds than the extraction in acetone and petroleum ether. The phytochemical analysis of aqueous extract of two plants studied showed almost similar results. Leaves of *ocimum sanctum* and

ocimum gratissimum both showed the presence of alkaloids, terpenoids, proteins, saponins, flavonoids, anthocyanin, phenols, carbohydrates and phytoserols. However Tannin was found to be absent in both stem and leaf extracts prepared in different solvents. The aqueous extract of ocimum sanctum stem showed the presence of terpenoids, proteins, phytosterol, phenol and carbohydrates whereas ocimum gratissimum stem showed the presence of alkaloids, protein, phytosterol, flavonoid, phenol, carbohydrate and anthocyanin . Alkaloids, saponins, flavonoids and tannin are absent in ocimum sanctum stem while ocimum gratissimum stem does not contain terpenoids, saponins and tannins.

Acetonic extracts of the leaves of both plants revealed the presence of alkaloids, terpenoids, phytosterols and phenols whereas the acetonic extracts of stem of the plants yielded different results. *O.sanctum* stem revealed the presence of terpenoids, proteins, phytosterols, flavonoids, phenol and anthocyanin and *O.gratissimum* stem had alkaloids, terpenoids, proteins and phenol. However, Phytosterols, Flavonoids, and Anthocyanin were completely lacking.

The leaf extracts in Petroleum ether shows positive response to the presence of terpenoids and phytosterols in case of O.sanctum and to terpenoids only by *O.gratissimum leaf. O.sanctum* stem extracts in petroleum ether has no response at all while *O.gratissimum* stem extracts responded to the presence of alkaloids and terpenoids.

In can be concluded that Acetonic extract of *Ocimum sanctum* leaves showed inhibition(8mm,11mm,8mm,9mm,10mm) against all the microorganisms selected for the study followed by Aqueous extract of *Ocimum Sanctum* leaves(6mm, 8mm, 9mm, 8mm, 8mm) .Petroleum ether extract of *Ocimum sanctum* leaves showed the least inhibition (6mm, no clear zone against Proteus vulgaris, 5mm, 5mm, 4mm). Acetonic extract of *Ocimum sanctum* leaves has high potential against Proteus vulgvaris, followed by *Streptococcus.aureus* (11mm, 10mm).

Acetonic extract of *Ocimum sanctum* stem showed inhibition (10mm,8mm,9mm,9mm,14mm) against all the microorganisms selected for the study followed by aqueous extract of *Ocimum Sanctum* stem (5mm,9mm,9mm,8mm,6mm) .Petroleum ether extract of Ocimum sanctum stem showed no inhibition. Acetonic extract of O.sanctum stem showed highest rates of inhibition against S.aureus and E.coli(14mm, 10mm).

Acetonic extract of *Ocimum gratissimum* leaves showed zone of inhibition against all the microorganisms selected for the study (12mm, 9mm, 10mm,12mm, 10mm) and maximum inhibition against *E,coli and Morganella*. In conclusion it can be said that Acetonic extract of *Ocimum sanctum* leaves showed highest inhibition zones,whereas both acetonic and aqueous extracts are equally good in case of Ocimum gratissimum leaves. Singh S et al. (2005).

Acetonic extract of *O.gratissimum* stem showed highest inhibition against Klebsiella and Morganella (14mm, 11mm) .With microbes ,Proteus and Streptococcus, both aqueous and acetonic showed similar results. However Petroleum ether showed no inhibition at all. Of these two plants, *O.gratissimum* showed more or equal antibacterial activity when compared to *O.sanctum* (Sharma and Bhadange, 2013).

The present study was aimed in the evaluation of preliminary phytochemical and antibacterial analysis of two species of Ocimum. Preliminary phytochemical investigation of two plants revealed the presence of several secondary metabolites whose quantification can be done by more advanced techniques and can used in the production of medicines .

CONCLUSION

The present study revealed the qualitative assay of several secondary metabolites in two species of Ocimum, Quantitative estimation of the different metabolites in these plants can be studied in future. The antibacterial properties of these plants may be due to one or several or a combined effect of these plant metabolites . Further studies in this area can undoubtedly led to the development of many new and novel drugs in future from these plants.

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