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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT, ANTIBACTERIAL ACTIVITIES OF *OCIMUM BASILICUM* L.

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Abstract

Tulsi leaves have been traditionally used for treatment of many infections. The present study in aimed at screening locally available plants such as Ocimum basilicum belonging to Lamiaceae family for its activities. The present study was undertaken to reveal the antioxidant and antibacterial activity of whole extract of O. basilicum against some human pathogen, identification of bioactive compounds through GC- MS and FTIR analysis. To study the phytochemical profile of the whole plant extracts. To study the phytochemical profile at the different extracts of O. basilicum. The whole plant crude ethanol extract characterised by GC-MS and FTIR analysis. The whole extract of O. basilicum and its antioxidant, antibacterial activities.

Keywords: O. basilicum, GCMS, FTIR, antioxidant, antibacterial

1. Introduction

Plant kingdom represents a rich source of organic compounds, many of which have been in use as agents against several infectious and non-infectious diseases, by the modern medicinal system. The World Health Organization country relies on traditional medicines, mostly plant drugs, for their primary health care needs (Loeraa et al., 2007). Particularly in rural India, use of raw plant products as well as some concoction of plant products in Ayurveda medicines is sought after to a great proportion, because of cheap availability, and in urban areas too those are increasingly popular for cultural nuances that exist (Sindhia, 2010). Further, a large number of phyto-drugs are popular and or rather harmless effects (Shahavi, 2008); almost all the viral infections are always addressed with plant product, as it is known. In ethno- botanical 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, 2009).

Medicinal plants do plays an important role in the treatment of ailments in Malaysia. The use of plant preparation for such purposes has been documented (Herbal Medicine Research Centre, 2002). More than hundred plant species in Malaysia are reported to have medicinal properties. Some of these plants are commonly used and have been used by people as folk medicine for hundreds of years (Herbal Medicine Research Centre, 2005). The control of bacterial infection has been remarkably effective since the discovery of antibacterial drugs. However some of the pathogens rapidly become resistant to many of the first discovered effective drugs. The development of drug resistance as well as appearance of undesirable side effects of certain antibiotics (WHO, 2002) has led to the search of new antibacterial agents in particular from medicinal plants. Higher plants have been shown to be a potential source for new anti-microbial agents .The screening of 166 plant extracts has been of great interest to scientist for the discovery of new drugs effective in the treatment of several diseases. A number of reports concerning the antibacterial screening of plant extracts of medicinal plants have appeared in the literatures.

Secondary plant metabolites

All edible plants in this study, occurs in the southern part of Iran and India. It is seems this plant contains high contents of anti-nutrients and phytochemicals. Plants are an essential component of the universe. Human beings have used plants as medicine from the very beginning of time. After various observations and experimentations medicinal plants were identified as a source of important medicine, therefore, treatment through these medicinal plants, began in the early stages of human civilization. In Islam diseases are cured in two ways, first the cure of soul through prayers and second the cure of ailments through medicines. Several phytochemical surveys have been published, including the random sampling approach which involved some plant accessions collected from all parts of the world. The major chemical substances of interest in these surveys have been the polyphenols and tannins, however, other diverse groups of naturally occurring phytochemicals such as alkaloids and saponins have also been reported. The natural active compounds classes or secondary metabolites as alkaloids, saponins, tannins and others have attracted researchers to investigate their chemical, toxicological and pharmacological features.

The alkaloids represent a group of natural products that has had a major impact throughout history on the economic, medical, political and social affairs of humans. They are a diverse group of low molecular weight nitrogen-containing compounds derived mostly from amino acids. These secondary metabolites are

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

found in about 20 % of plant species and they classified as true alkaloids, A wide range of biological activities of alkaloids have been reported: emetic, anti-cholinergic, antitumor, anti-diuretic, sympathomimetic, antiviral, antihypertensive, hypnoanalgesic, antidepressant, miorelaxant, antitussigen, antimicrobial and anti-inflammatory. However, the alkaloids and other natural compounds have complex activities and it is necessary to analyze pharmacological activities in the general tissues, linking the structure with the activity presented. It is common to find pharmacological results where a single experimental model generalizes a biological answer, but these can't be accepted because all the pathologies in question are also complex and it is necessary to investigate specific experimental models (Abulude, 2007).

Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity (Hotwani, 2014). Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities (Sala, et al., 2003). The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing (Ashokkumar ,2008&Veerapur ,2009) and in recent years, there has been a worldwide trend towards the use of the natural phytochemicals present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables (Kitts 2000&Wang , 2000). In recent years, secondary plant metabolites (Phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krshinaraju, 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrin MF, 1985.). Pteridophytes are not infected by microbial pathogens, which may be one of the important factors for the evolutionary success of pteridophytes and the fact that they survived for more than 350 million years. Considering the rich diversity of Indian medicinal plants including Pteridophytes, it is expected that, the screening of plant extract for antibacterial activity may be beneficial for humans and plants diseases (Sharma BD, 1985).

Infectious diseases accounts for high proportion of health problems in the developing countries including India. Microorganisms have developed resistance to many antibiotics and as a result, immense clinical problem in the treatment of infectious diseases has been created. The resistance of the organisms increased due to indiscriminate use of commercial antimicrobial drugs, commonly used for the treatment of infectious disease. This situation forced the researchers to search for new antimicrobial substance from various sources including medicinal plants. Keeping in view, the importance of medicinal value of plants. The study of biologically active compounds from natural sources has always been of great interest to scientists looking for new sources of useful drugs for treating infectious diseases. Infectious diseases caused by bacteria, fungi, viruses, and parasites remain a major threat to public health, despite tremendous progress in human medicine. Their impact is particularly great in developing countries because of the relative unavailability of medicines and the emergence of widespread drug resistance (Nirater et al., 2015).

Bacteria cause serious infections in humans as well as other animals. For example, it was found that Staphylococcus aureus (S. aureus) causes superficial skin lesion and food poisoning 3. Pseudomonas aeruginosa (P. aeruginosa) is a nosocomial pathogen accounting for a significant percentage of hospital-acquired infections and health care centers because there are a little effective antimicrobial agents against it (Abu-Shanab et al., 2004). Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines which have made large contributions to human health and well-being. Although many drugs that come from trees generally have been replaced by more potent synthetic ones, trees remain a source for some drug ingredients (Thomson WAR. 1978). Medicinal plants have become important for the treatment of different disease conditions, such as diabetes, malaria, anemia for a long time now (Fola A. 1993) but the potential of higher plants as source for new drugs is still largely unexplored (Gerharts W. 1985) Systematic screening of them may result in the discovery of novel effective compounds (Tomoka et al., 2002). Many plants, spices and herbs are sources of various bioactive substances and therapeutic compounds, which are essential for maintaining the human health. These substances provide defensive mechanism to plants against predation by microorganisms, pests, insects and herbivores (Tapsell, 2006).

Plants are fundamental source for all other living organisms. During the evolution process plants represent the first stage and they produce the most important materials such as nutrients, fuel, oxygen, etc. Higher plants also play a dominant role in the maintenance of human health by producing many bioactive compounds. Green plants represent a reservoir of effective chemo therapeutants that are easily biodegradable, systemic and non-phytotoxic (Verma, 2006). The crude leaf extracts of Tulasi with two solvents, methanol, acetone, and water (polar to non-polar, extracted by both cold and hot extractions) were used to monitor antibacterial property against 5 clinically isolated MDR bacterial strains (Staples, 1999). Tulasi is cultivated for religious and medicinal purposes, and for its essential oil. It is widely known across the Indian

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

Subcontinent as a medicinal plant and an herbal tea, commonly used in Ayurveda, and has an important role within the Vaishnavite tradition of Hinduism, in which devotees perform worship involving holy basil plants or leaves.

The variety of Ocimum tenuiflorum used in Thai cuisine is referred to as Thai holy basil it is not to be confused with Thai basil, which is a variety of Ocimum basilicum. Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries. The discovery of medicinal plants in different parts of the world is important both to the agriculture and medicine sectors, in establishment of new directions towards propagation of alternative medicinal crops that offer better economic and social benefits.

Chemical properties of Ocimum basilicum

Different components such as coffee acid, p-coumaci acid, mycenae, rutin, Tryptophan are used in the leaves of O. basilicum, leaf. These are used as remedies for disorders such as viral, ocular, respiratory and hepatic infections Ocimum bacilicum leaf. There are used most important of all Ocimum species which are all together commonly referred to as basil (Sobti and Pushpangadan et al., 1997). O. basilicum leaf is also known as sweet basil and king of the species. It is a popular chinese medicinal hrub of the family capital. The whole plant materials and essential oils have used extensively in food, perfumes, dental and oral products (De Feo and Senatore, 1993). The leafs and flowing parts of these plants are antispasmodic, aromatic, carminative, digestive, stomachic and stimulant they are administered orally in the treatment of fever, poor digestion, nausea, depression, and exhaustion.

Biological activities of Ocimum basilicum

Ocimum basilicum (Fig 1) is often used as effective medicine worldwide (Tchoumbougnang et al., 2006). In many countries, this herb is widely used for culinary purposes spice, flavors, essential oil and therapeutic application (Bilal et al., 2012). The leaves and flowering parts of O. basilicum are commonly used to treat fever, nausea, abdominal cramps, gastroenteritis, migrins, insomnia, depression, gonorrhea, dysentery, chronic diarrhea and exhaustion. External applications include treatment of acne, loss of smell, insect stings, snake bites and skin infection (Kaya et al., 2009). More importantly, have been identified for its profound anti-lipidemic, anti-cholesterol, anti-microbial and anti-diabetic properties (Benalla et al., 2012). Safety, therapeutic effectiveness, economic benefits and availability are important advantages that O. bacilicum possess (Dinesh Kumar et al., 2010). In addition, their bioactive compounds offer numerous additive health benefits (Nickavar and Yousefian, 2012) and provide a natural source of anti-oxidants capable of neutralizing free radical and reducing the severity of diabetic micro and macro-vascular complications (Swarnalatha et al., 2012).

Figure 1. Ocimum basilicum

The essential oil of this plant is used as a food additive and in cosmetics (Prakesh et al., 1990). O. basilicum extracts have been used to treat headaches, cough, diarrhea, warts, constipation, kidney mal-functions and antimiuobial infections (Simon et al., 1999). Siddha system is dravidian version of Indian medicine developed by ancient Tamil sages called as siddhacs. Siddhas were great scientist in ancient times.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

The Siddha system of medicine has been practiced for over 5000 years throughout Tamil Nadu, India (Fritts et al., 2008).India being rich in herbs, can utilize its herbs for such purpose plant not only being antimicrobial and antifungal, they can also act as an effective anticancer, antiparasitic and antimalarial agents. Then it has been expensively exploited for these properties, here in the present study, we used the plant source O. basilicum and silver nanoparticles as antioxidant and antimicrobial activities. The large number of plant are used other skin diseases (Sankarnarayanan et al., 2010). In indigenous system of medicine, it is reported that leaves were used for skin diseases, rheumatism and as analgesic (Manikandar et al., 2006). Hence the present study was undertaken to reveal the antioxidant and antibacterial activity of whole extract of O. basilicum against some human pathogen, identification of bioactive compounds through GC- MS and FTIR analysis.

2. MATERIALS AND METHODS

Plant collection

Ocimum basilicum weeds are found growing as dense clumps along the roadsides of Kanchipuram, Tamilnadu, India. O. basilicum was identified by Dr. P. Paramasivam, Department of Botany, Pachaiyappa's College for Men, Kanchipuram. The voucher specimen was numbered and deposited in our botany herbarium.

Preparation of plant extracts

The dried plant was powdered and sieved to get fine powder using an electric blender. 70 g of the plant powder was filled in the thimble and extracted successively with aqueous, acetone chloroform, ethanol and methanol soxhlet extractor for 10 h. All the extracts were concentrated using rotary flash evaporator and preserved at 5˚ C in airtight bottle until further use.

Phytochemicals screening

The phytochemical screening was carried out as described by (Nazer et al., 2009; Senthilkumar and Reetha, 2009).

Test for carbohydrates

Molisch's Test: To 2 ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Formation of purple or reddish ring indicates the presence of carbohydrates.

Test for Tannins

Ferric Choloride Test: To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence for tannins.

Test for saponins

Foam Test: To 1ml of plant extract, 5ml of distilled water was added and shaken in a graduated cylinder for 15 min length wise. Formation of 1cm layer of foam indicates the presence of saponins.

Test for flavonoids

Sulphuric Acid Test: A fraction of the extract was treated with concentrated sulphuric acid and observed for the formation of orange colour.

Test for alkaloids

Mayer's Test: To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Formation of green colour or white precipitate indicates the presence of alkaloids.

Test for quinines

Sodium Hydroxide Test: To 2 ml of plant extract, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100 C, Formation of bluish green colour indicates the presence of anthocyanin and formation of yellow colour indicates the presence of betacyanin.

Test for quinines

Sulphuric Acid Test: To1 ml of extract, 1 ml of conc. H_2SO_4 was added. Formation of red colour indicates the presence of quinones.

Test for glycosides

Sulphuric Acid Test: To 2 ml of plant extract, 1 ml of glacial acetic acid and 5 % ferric chloride was added to which few drops of concentrated sulphuric acid were added. Presence of greenish blue colour indicates the presence of glycosides.

Test for cardiac glycosides

Ferric Chloride Test: To 0.5 ml of extract, 2 ml of glacial acetic acid and a few drops of 5% ferric chloride were added. This was under layered with 1 ml of conc. H_2SO_4. Formation of the brown ring at the interface indicated presence of cardiac glycosides.

Test for Terpenoids

Sulphuric Acid Test: To 0.5 ml of extract, 2 ml of $CHCL_3$ and conc. H_2SO_4 was added carefully. Formation of red brown colour at the interface indicated presence of terpenoids.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

Test for Triterpenoids

Libermann-Buchard test

To 1.5 ml of extract, 1 ml of Libermann-Buchard reagent (acetic anhydride and conc. H2SO4) was added. Formation of bluish green colour indicated presence of triterpenoids.

Test for phenols

Ferric Chloride Test: To 1 ml of the extract, 2 ml of distilled water was added followed by few drops of 10% ferric chloride. Formation of blue or green colour indicated presence of phenols.

Test for cumarins

Sodium hydroxide Test: To 1 ml of the extract, 1 ml of 10% NaOH was added. Formation of yellow colour indicates the presence of cumarins.

Test for proteins

Ninhydrin Test: To 2 ml of plant extract, few drops of 0.2% ninhydrin was added and heated for 5 min. Formation of blue colour indicates the presence of proteins

Test for steroids and phytosterols

Sulphuric Acid Test: To 1 ml of plant extract, equal volume of chloroform and few drops of concentrated sulphuric acid were added. Formation of brown ring indicates the presence of steroids and formation of bluish green colour indicates the presence of phytosterols

GC-MS analysis

GC-MS analysis of the crude of whole plants were carried out on Agilent technologies (6890 N), JEOL GCMATE II which comprised of an auto sampler and gas chromatography interfaced to a mass spectrometer (GC-MS) instrument exploying the following condition: capillary column – 624 ms (30 m x 0.32 mm x 1.8 m) operating in an electron mode at 70 eV; helium (99.999 %) was used as carrier gas at a constant flow of 1.491 ml/min and injection volume of 1.0 ml, injector temperature was 140° C Mass spectra were taken at 70 eV.

Antioxidant activity

The method described by Oyedemi et al., (2011) was used to determine DPPH scavenging activity of the plant extract. The solution of 0.135mM DPPH was prepared in methanol. Different concentration of extract such as 10, 20, 40, 60, 80 and 100 µg was mixed with 3 ml of DPPH solution. The reaction mixture was vortexed thoroughly and left in the dark are room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as the reference drug. The ability of plant extract to scavenge DPPH radical was calculated from the following formula:

%DPPH inhibition= [(OD of control - OD of test)/ (OD of control)] x100

Anti-bacterial activity (Well-diffusion method)

The antibacterial activities of all extracts were carried out by well diffusion method. The concentrations of the test compounds were taken in DMSO and used in the concentration of 25, 50, 75 and 100 µg. The target microorganisms were cultured in Mueller–Hinton broth (MHB). After 24 hr the suspensions were adjusted to standard sub culture dilution. The Petri dishes containing Muller Hinton Agar (MHA) medium were cultured with diluted bacterial strain. Well made of diameter 6 mm was pre-sterilized and was maintained in aseptic chamber. Each concentration was injected to the sterile well papers. Then the prepared wells were placed on the culture medium. Standard drug streptomycin (10µg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 37 ºC for 24 hr. The diameter of the clear zone around the well was measured and expressed in millimeters as its anti-bacterial activity.

Organisms used

S.no	Organisms	Type
1.	**Bacillus subtilis**	**Gram-positive**
2.	**Escherichia coli**	**Gram-negative**

Statistical analysis

Analysis of variance (ANOVA) was carried out using SPSS program (version. 15). Means and standard deviation of three replicates were calculated. The results were considered significant at $p < 0.05$.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

3. RESULTS

Preliminary screening of active phytochemicals

Table 1. Phytochemical screening of whole plant ethanol extract of the O. basilicum

S. No	Secondary metabolites	Aqueous	Acetone	Ethanol	Hexane
1	Carbohydrate	+++	+++	+++	+
2	Tannins	+	+	++	-
3	Saponins	+++	+	+++	++
4	Flavonoids	+	++	+++	-
5	Alkaloids	-	-	++	+
6	Anthocyanin	+	-	+++	-
7	Quinones	-	+	++	-
8	Glycosides	-	-	-	-
9	Cardiac glycosides	++	+	+++	++
10	Terpenoids	++	+	+++	-
11	Triterpenoids	++	+	++	-
12	Phenols	+++	+	+++	++
13	Coumarins	-	+	-	-
14	Acids	-	-	-	+
15	Protein	-	-	-	-
16	Steroids	++	++	+++	+++

+++ Strongly positive ++ Positive + Trace - Not detected

Preliminary phytochemical screening of revealed the O. basilicum presence of phytochemicals such as tannins, alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, saponins, steroids, terpenoids and triterpenoid. Quinones, coumarins, cyanin and glycosides are absent.

Antioxidant activity of whole plant ethanol extract

The DPPH free radical scavenging activities of the whole plant ethanol extract of O.basilicum increased with increasing concentrations. The DPPH radical scavenging activities of ethanol extract of O.basilicum and ascorbic acid were in increasing (Fig.2).

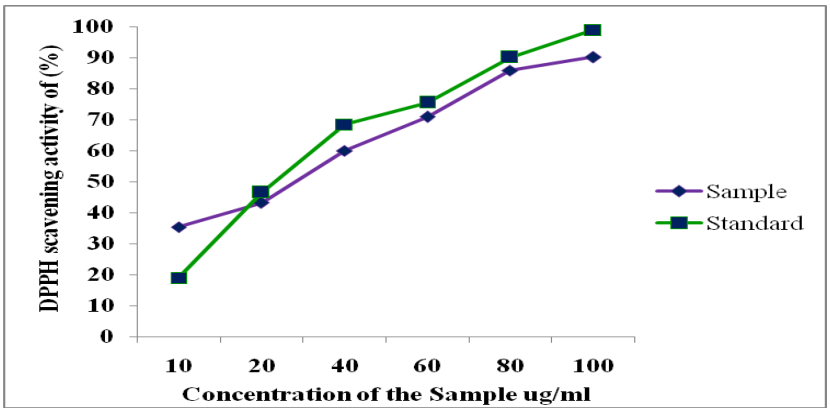

Figure 2. Antioxidant activity of ethanol whole plant extract of O.basilicum

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

FTIR analysis of ethanol extract

The infrared spectrum of the crude ethanol while plant extract (Fig.3) was typical of a amides compound with a broad band between3420 cm^{-1}. A pronounced signal at 744 cm^{-1} stretch aliphatic amines. The peaks at 2923 cm^{-1} are due to C-H stretch of alkenes and a Peak at 1652 cm^{-1} due to C=O stretch at ketones groups. The medium absorption peak located at 1455 cm^{-1} are due to C-H bend of alkenes and a peak at 1265 cm^{-1} due to N-H stretch aromatic amines and a peak at 1180 cm^{-1} assigned to C-H wag stretch alkylhalides and a peak at 1049 cm^{-1} due to C-N stretch aliphatic amines.

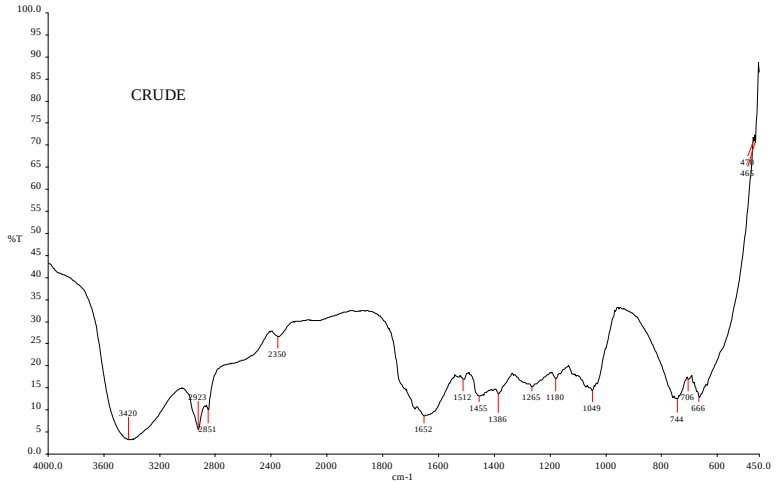

Figure 3. FTIR analysis of whole plant ethanol extract

GC-MS analysis of whole plant ethanol extract

The composition and identification of the main compounds present in the ethanol extracts of O.basilicum is shown in (Table. 2). Twelve compounds were identified by GC-MS. The main compounds were Nephthalene Androsta-1-4 diene-3,11,17-trione Phytol Geranyl is ovalerate 2-pentadeanone,6,10,14-trimethyl Longifolenaldehyde Eucalyptol Dihydrocarveo methyl tetradecanate, eicosanoic acid, methyl ester, 1,2-benzenedicarboxylic acid, octadcaboic acid (Fig. 4).

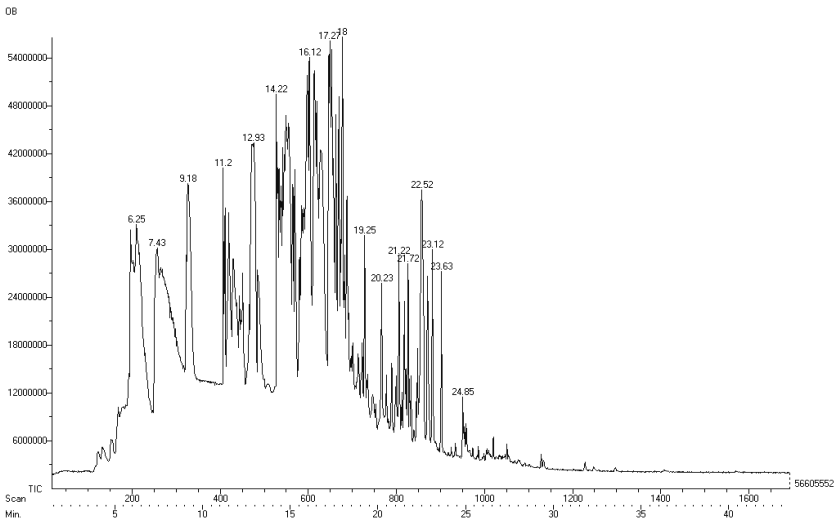

Figure 4. GCMS analysis of whole plant ethanol extract

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

Table 2. Phtotcompounds identified from whole plant ethanol extract of O.basilicum by GCMS

S.no	Name of the compound	Retention time	Molecular formula	Molecular weight g/m
1	Nephthalene	12.93	$C_{10}H_8$	128.1705
2	Androsta-1.4 diene-3,11,17, trione	23.13	$C_{19}H_{22}O_3$	298.382
3	Phytol	22.52	$C_{20}H_{40}O$	296.539
4	Geranyl iso valerate	21.22	$C_{12}H_{26}O_2$	238.3657
5	2-pentadeanone,6,10,14,trimethyl	19.25	$C_{15}H_{30}O$	226.404
6	Longifolenaldehyde	18	$C_{15}H_{24}O$	220.356
7	Eucalyptol	6.25	$C_{10}H_{18}O$	154.249
8	Azulene,1,2,3,4,5,6,7,8-octahydro-1,4,-dimethyl-(1-methylethylidene)	17.27	$C_{10}H_8$	128.174
9	Isoaromadendrene epoxide	16.12	$C_{15}H_{24}O$	220.356
10	Dihydrocarveo	7.43	$C_{10}H_{18}O$	154.253
11	Bicyclo(3,1,0) hexane-6-methanol ,2-hydroxy-1,4,4-trimethyle	11.2	C_6H_{10}	82.146
12	9-methoxybicyclo(6.1.0) nono-2,4,6,-triene	9.18	$C_{11}H_{18}O_s$	190.262

Antimicrobial activity of O.basilicum

Antimicrobial activity of plant extract against Gram negative (Escherihia coli) and Gram positive Bacilles Subtilies) bacteria revealed an antimicrobial activity against the test microorganisms. The zone of inhibition of plant extract against gram negative and gram positive bacteria was measured. The result indicated that from O.basilicum whole plant extract showed effective antibacterial activity both gram negative and gram positive bacteria (Fig 5). The antimicrobial effective of the plant extract was examined using the well diffustion assay which is mainly used to test the sensitivity of bacterial strains towards antibiotics with a clear zone around the well reflects he bacterial sensitivity forward antibiotics (Table 3 and 4).

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

Table 3. Size of inhibition zone of whole plant extract with different solvent of O. basilicum against bacteria

Sample code	Zone of inhibition (mm) µg/Ml							
	Bacillus subtilis				Escherichia coli			
	25 µg	50 µg	75 µg	100 µg	25 µg	50 µg	75 µg	100 µg
Hexane	-	-	-	-	-	-	-	-
Acetone	-	-	-	-	-	-	-	-
Ethanol	8	9	11	11	9	11	12	12
Aqueous	-	-	-	-	-	-	-	-
Streptomycin (10µg)	21				22			

- = Not active

Table 4. Size of inhibition zone of whole plant extract with different solvent of O. basilicum against bacteria

Sample code	Zone of inhibition (mm) µg/mL			
	Candida albicans			
	25 µg	50 µg	75 µg	100 µg
Hexane	-	-	-	-
Acetone	-	-	-	-
Ethanol	18	19	19	21
Aqueous	-	-	-	-
Clotrimazole (10µg)	20			

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

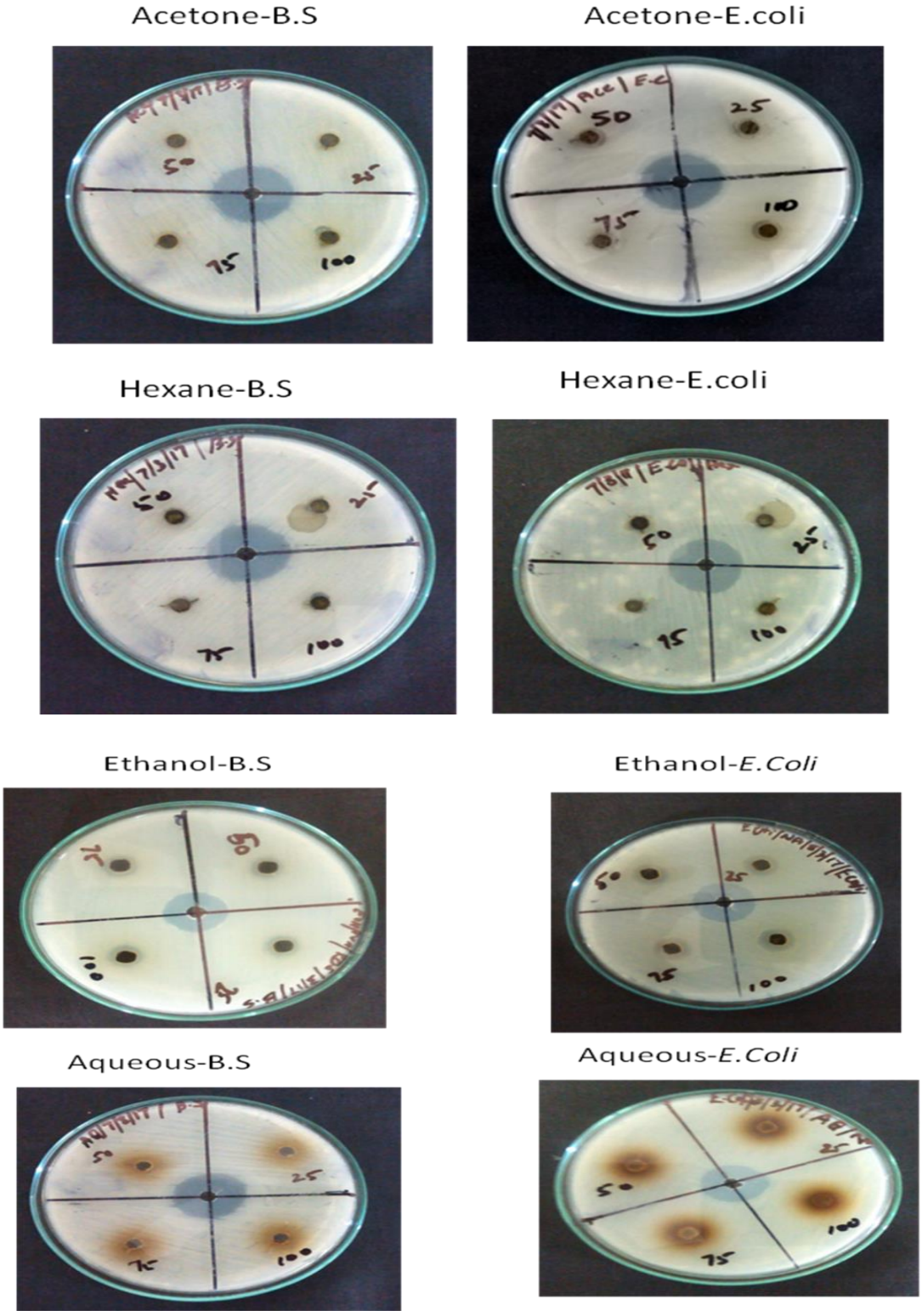

Figure 5. Antibacterial activity of whole plant different extracts of O.basilicum

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 01, 2021

4. DISCUSSION

Phytochemical compounds may be the bioactive constituents responsible for the efficacy of the leaves of the plants studied. The presence of some of these compounds has also been confirmed to have antimicrobial activity. Hence it could be inferred that the plant extracts could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection. The results confirm the presence of constituents which are known to exhibit medicinal as well as physiological activities (Mukeshwer Panday, et al., 2011). The phytochemical characteristics of the whole plant extract of Ocimum basilicum investigated are summarized. The results reveal the presence of medicinally active constituents like tannins, alkaloid, terpenoids, steroids and flavnois, phlobatannins, glycosides in the leaves of Ocimum basilicum. The phytochemical screening of flowers and flower buds are not been reported earlier although flower and flower buds of M. conanensis also help in abortion and leucorrhea (Anbazhakan et al., 2007). Alkaloids have been used as both antibacterial and antidiabetic Properties and phenolic compounds have been extensively sed in disinfections and reman the standard with which other bacterisides are compared (Akinyeye et al., 2014).

The alkaloids contained in plants are used in medicine as anesthetic agents (Victor Njoku O. and Chidi Obi, 2009). The presence of saponins in plants has been reported to be responsible for the tonic and stimulating activities observed in Chinese and Japanese medical herbs. The results obtained in this study thus suggest that the identified. Ethanol extract from both dry and fresh bark extracts were more effective against S.aureus compared to water extracts. These results are the same as the ones obtained in study carriedout by Gajendrasinh et al. (2012) . In all cases, ethanol extracts were the best in terms of effectiveness against S.aureus. According to the report done by Kirtikar and Basu(1975), whose results are similar to the ones we obtained, they said that the active ingredients are slightly soluble in water and freely soluble in organic solvents such as alcohols,these ingredients include: azardirachtin, 1-maliantriol, salannin, nimbin, nimbdin and others. Our results also confirm the information reported by Ibekwe et al. (2001) wherem by ethanol extracts were more effective than water extracts.

The petroleum ether and chloroform extracts were found active against Pseud. aureginosa and the two fungi, C. albicana and Aspergillus niger. This result is similar to that reported by Yazdani et al. (2009) who found that the extracts of anise seeds inhibited only dermatophyte species, while extracts of star anise fruits inhibited growth of all dermatophytes and saprophytes tested. The high antifungal activity of P. anisum is probably due to the high concentration of an ethanol in the extract or as a result of the synergism of its components. Vat et al. (2011) reported that the chloroform extract of roots of Murraya koenigii (Linn.) Spreng. (Rutaceae) showed good inhibitory properties against A. niger, P.aeruginosa, and C. albicans and even at low concentrations and the Petroleum ether is inhibitory against A. niger and P.aeruginosa.

Result of phytochemical screening of Olea europea leaves us of the various extracts showed the presence of saponins, stérols, steroid, terpen and flavonoids. All these compounds were previously reported to occur in olive leaf 19-20The extracts of leaves of guava (Psidium guajava) and cloves (Syzgium aromatica) showed inhibitory effects on growth of S. aureus, with inhibition zones ranging from 10 to 20 mm and from 21 to 30 mm, respectively 24.The present investication shows that sisgnifiant variation in the content like alkaloids, flavonoids, phenol and carbohydrate when compard to above mentioned results. These Variation are due to number of environment factor such as climate, altitude, eainfall etc. As mentioned (Kokate et al.,2004). Saponins act as antimicrobial activity and extremel to Coldblooded animals, but toxicity to mammals is low (Sneh verma et al., 2013) Sapnonins Are a mild detergent used in intracellular histochemistry staining to allow antibody access to intracellular proteins. The sapnonin are used in hypercholestrolaemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory activity and weight loss (Manickam murugan et al., 2014). Doughari et al. (2007), reported the anti-bacterial effect of the root extract of C. papaya on various bacterial isolates including B. cereus. The presence of Saponin supports the fact that pawpaw flower has cytotoxic affect such as permenlization of the intestine as saponin are cytotoxic (Okwu and Okwu, 2004). Alkaloids are the most efficient therapeutically significant plant substance. Pure natural and synthetic derivatives of alkaloids are used as a basic medical agent because of their analgesic, antispasmodic and antibacterial properties (Stray, 1998). The presence of Alkaloid in the flower shows that this plant can be effective anti-malaria agent since alkaloid consists of quinine, which is anti-malaria (Robinson, 1995).

Subapriya and Nagini 7reported that presence of high concentrations of azadirachtins, quercetin and β-sitosterol in A. indica leaves might be responsible for strong antibacterial and antifungal activity. Furthermore, Maragathavalli and his co-authors 8 studied the antimicrobial activities of ethanolic extracts of Neem leaves in various concentration against pathogenic bacteria and comparedthat to gentamycin. They found that the ethanol extract showed maximum inhibition on Bacillus pumillus, Pseudomonas aeruginosa and Staphylococ cus aureus in an ascending order. In the existing literature, berberine has been reported to be produced by nmeros Berberis spp. Including, B.nepalensis B.asiatia and B.lycium (Chandra and

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Purohit,1980), B.aetnensis (Iauk et al., 2007), B.stolonifera (Stadler et al., 1988), B.chitria (Hussaini and Shoeb, 1985).

The most active ingredient of B.aristata is berberine, a quaternary isoquinoline alkaloid and the content of berberine is used as biomarker of the plant. It is mostly found in the roots, rhizomes and stem bark (Pasrija et al., 2011). Native berberine has already been reported to possess antimicrobial activities against a wide variedy of microorganisms including Gram-positive and Gram-negative.

CONCLUSION

The present study and data revealed that the antimicrobial activity of leaf extract of O. basilicum was found to be the best against bacterial, and also proved that the factors which are found in the form of secondary metabolites were responsible for antimicrobial activity. The future prospect for this study was to analyze the purified compound for drug validation. However further research is needed in-vitro as well as in -vitro and the extracts against other different types of microorganism to reach a better conclusion.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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