Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2582-2845.7876

ISSN: 2582 – 2845

Ind. J. Pure App. Biosci. (2019) *7*(6), 192-202

Research Article

Qualitative and Quantitative Phytochemical Analysis on Ocimum Species of Karnataka

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Received: 6.11.2019 | Revised: 2.12.2019 | Accepted: 7.12.2019

ABSTRACT

Ocimum species is a fast-growing shrub belonging to the family Lamiaceae. The various species of Ocimum such as Ocimum basillicum, Ocimum gratissimum, Ocimum kilimandscharicum, Ocimum sanctum(green), Ocimum sanctum(purple) have found its great role in pharmacology. In this study, Qualitative and Quantitative phytochemical analysis has been done to determine the presence of phytochemical compounds such as Alkaloids, Flavonoids, Saponins. Tannins, Phenols, Proteins, Cardiac glycosides, Terpenoids, Carbohydrates, Quinones by using standard methods in the different extracts of Ocimum leaf powder. Determination of all these secondary metabolites content has been carried out. The secondary metabolites obtained from these plants act as natural antioxidants which have medicinal values to humans. Thus in our present study, Qualitative and Quantitative phytochemical analysis has been done in the leaves of Ocimum plants which is used in the pharmacology at greater use.

Keywords: *Ocimum basilicum, Ocimum gratissimum, Ocimum kilimandscharicum, Ocimum sanctum, phytochemical, Antioxidants, Qualitative-Quantitative analysis.*

INTRODUCTION

The secondary metabolites known to be present in plants as complex synthesising materials are also known as phtochemicals. Plants produces biologically active compounds known as phytochemicals for fighting against diseases. It is reported in various research works that due to their antioxidant activity, Phytochemicals has the ability to fight against diseases (Farombi et al., 1998; Halliwell et al., 1992). The plant parts like leaves, flowers, stems, roots, seeds, fruit and bark from centuries are known for its medicinal value due to the presence of phytochemicals like Alkaloids, Tannins, flavonoids and phenolic compounds (Hill, 1952).

The various species of Ocimum such as *Ocimum basillicum, Ocimum gratissimum, Ocimum sanctum* (green), Ocimum sanctum (purple), *Ocimum kilimandscharicum* belonging to the family "Lamiaceae=Labiateae" has great medicinal values across the worldwide with its properties like antioxidant and antibacterial activity.

Cite this article: Pavithra Kumari, H.G., & Narase Gowda, P.N. (2019). Qualitative and Quantitative Phytochemical Analysis on Ocimum Species of Karnataka, *Ind. J. Pure App. Biosci. 7*(6), 192-202. doi: http://dx.doi.org/10.18782/2582-2845.7876

Ocimum basillicum is known by its common name Basil, mostly occur in the East Anatolia region in Turkey (Adiguzel et al., 2005). The flavoring used in meats and sausages are the essential oils extracted from *Ocimum basillicum*. The *Ocimum basillicum* is having great medical importance wherein its leaves and flowers are used to treat fever, nausea, abdominal cramps, gastroenteritis, migraine, insomnia, depression, Gonnorhoea, dysentery which can pave the way for other uses such as anti-spasmodic, aromatic, Carminative, digestive, galactagogue, stomachic, tonic agents (Chiej, 1984; Duke et al., 1985).

Ocimum gratissimum commonly known as Clove basil, sweet basil, tea bush, scent leaf or fever plant, is an herbaceous shrub found in Nigeria. The essential oil of *Ocimum gratissimum* has main component Eugenol which has inhibitory action on Haemonchus contortus (Pessoa et al., 2002; Hussien et al., 2011) used in treating epilepsy, shigellosis (Idika, 2008) which is also used in treating Asthma, Pneumonia, bronchiolitis, Urogenital infections, skin infections such as Dermatitis, eczema, scabies (Adjanahoun et al., 1991; Elujoba et al., 2005). *Ocimum gratissimum* has been used to protect various plants cereals and legumes during storage against pest diseased by peasant farmers of northern Nigeria (Mann et al., 2003).

Ocimum sanctum has got its common name as "Holy Basil" or Tulsi. It has two varieties of Shri Tulsi with green leaves (Prakash et al., 2005) and with purple leaves as Krishna Tulsi (Sebastian Pole, 2006). Eugenol is the main constituent present in the essential oils of these plants. It has its importance in a pharmacological activity such as expectorant, analgesic, hypolipidemic which is used in the treatment of Fever, arthritis, convulsions, bronchiolitis, etc.

Kilimanjaro, Kenya (East Africa) and also widely scattered in the regions of Rwanda, Athens, Nigeria, Ghana, Thailand, India. Ocimum is mainly diversified in Africa (Paton, 1992), South America (Brazil), Asia (India) (Pushpagandan et al., 1995; Sobti et al.,1982). They vary from 30 to 160 species (Pushpagandan et al., 1995). Ocimum has 68 species names accepted out of 333 scientific plant names whereas rest are documented as unassessed, unplaced and synonyms (Anonymous 2014). Ocimum has represented India by its 9 species which are mainly found in the tropical and peninsular region (Anonymous, 1966). It has mainly got its importance in Pharmacology by its therapeutic properties.

Therefore, our present work focuses on the Phytochemical constituents present in the leaf extract of various species of Ocimum and their uses in the field of pharmacology.

QUALITATIVE PHYTOCHEMICAL ANALYSIS:

MATERIALS AND METHODS

The plant samples of different species were obtained from the University of Agriculture and maintained in the greenhouse of Visveshwarapura College of Science, Bangalore India. The obtained leaf samples were cleaned with distilled water, dried under shade, powdered and stored in airtight bottles.

A. Solvent extract preparation:

50ml of methanol is used to extract 5g of each powdered sample for 48hours. After 48hours the supernatant obtained was used to make the crude extract by the process of evaporation.

The preliminary phytochemical analysis was used to analyze the presence of compounds namely Alkaloids, Flavonoids, Saponins, Tannins, Phenols, Proteins, Cardiac glycosides, Terpenoids, Carbohydrate and Quinones (Solomon Charles Ugochukwu et al., 2013).

Determination of alkaloids

Wagners reagent were added in few drops to 2ml of methanol and ethanol extracts, the reddish brown precipitate showed the presence of alkaloids.

Determination of flavonoids:

The yellow color was observed when 20% of NaoH was added in few drops to 2ml of each extract. To this, 70% dilute Hcl was added in few drops and the yellow color disappeared. The flavonoids presence were determined by the formation and disappearance of yellow color.

Determination of Saponins

2ml of each extract was mixed with 6ml of distilled water and shaken vigorously. Saponins were determined by the appearance of bubbles or foam.

Determination of tannins

Alchoholic Ferric chloride was added in the concentration of about 10% to 1ml of each extract, Tannins were determined by the appearance of blue or black color.

Determination of phenols

1ml of 5% aqueous ferric chloride was added to 1ml of each extract, Phenols were determined by the appearance of blue color in the extract.

Determination of proteins

1ml of 40% NaoH and a few drops of 1% copper sulphate was added to 2ml of each extract. The peptide linkage molecule in the extracts were determined by the appearance of violet color.

Determination of cardiac glycosides

0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric hloride was added to 1ml of each extract. The cardiac glycosides in the extract were determined by the appearance of brown ring at the interface.

Determination of Terpenoids

0.5ml of chloroform and a few drops of concentrated sulphuric acid was added to 1ml of each extract. The terpenoids were determined by the appearance of reddish brown precipitate.

Determination of carbohydrates

Molischs reagent were added in few drops and 1ml of concentrated sulphuric acid was added along the sides of the test tubes to 1ml of each extract. The mixture was allowed to stand for 2-3minutes. The appearance of red or dull violet color determined the presence of carbohydrates in the extract.

Determination of Quinones

The concentrated Hydrochloric acid was added to 1ml of each extract. The Quinones were determined by the appearance of yellow precipitate.

RESULTS AND DISCUSSION

QUALITATIVE ANALYSIS:

The phytochemical analysis has been done in the different species of Ocimum plants by subjecting them to various phytochemical evaluation using methanol extract. The results of the phytochemical screening carried out for various chemical constituents with methanol extract is shown in table 1. The presence or absence of different constituents with respect to methanolic extract is shown in the below table. All phytochemicals except phenols were found to be present in methanolic extract of few Ocimum species sample which is selected to study here. Phenols are absent in all the species whereas all the other compounds are present in all the species except in Ocimum gratissimum and Ocimum sanctum (purple) where tannins are absent.

All Ocimum species have high phytochemicals content except phenols. Presence of phytochemicals like flavonoids, saponins, tannins in Ocimum species indicates the high antioxidant activity. These phytochemicals are natural oxidants which prevent essential oils from oxidative stress. Ocimum sanctum (purple) and Ocimum gratissimum showed the absence of phenols but still, they have higher antioxidant activity with the other phytochemicals. According to other works of literature, the presence of phytochemicals like alkaloids, tannins, flavonoids, and phenolic compounds has been studied (Hill, 1952). and it has been shown that these phytochemicals have higher antioxidant activity (Farombi et al, 1998; Halliwell et al., 1992). From our present work, it is clear that all the phytochemicals extracted from methanolic extract of leaves of Ocimum species showed higher antioxidant activity.

Table 1: Qualitative Phytochemical Analysis of *Ocimum* samples

Qualitative Phytochemical Analysis of *Ocimum species*					
Test	*Ocimum basillicum*	*Ocimum gratissimum*	*Ocimum kilimandscharicum*	*Ocimum sanctum green*	*Ocimum Sanctum purple*
Carbohydrates	+	+	+	+	+
Quinones	+	+	+	+	+
Proteins	+	+	+	+	+
Terpeniods	+	+	+	+	+
Flavonoids	+	+	+	+	+
Saponins	+	+	+	+	+
Tannins	+	-	+	+	-
Cardiac Glycosides	+	+	+	+	+
Phenol	-	-	-	-	-
Alkaloids	+	+	+	+	+

All phytochemicals except phenols were found to be present in Methanolic extracts of selected *Ocimum sp.*sample

i. *Ocimum* sp. Samples Extracts

QUANTITATIVE PHYTOCHEMICAL ANALYSIS:

MATERIALS AND METHODS

The quantitative assay was done based on the results of qualitative method for the determination of Alkaloids, Tannins, Phenols, Flavonoids, Saponins, Proteins and Carbohydrates.

Total Tannins Content Determination:

Folin and Ciocalteu method was slightly modified for the determination of Tannins. 7.5ml of distilled water, 0.5ml of Folin Phenol reagent, 1ml of 35% sodium carbonate solution was added to 1ml of sample extract. The absorbance was read by 725nm. 0 to 0.5mg/ml of Tannic acid dilutions were used as standard solutions. The Tannic acid is expressed in terms of mg/ml of extract to determine the results of tannins (Sazzad Hossain1 et al., 2013).

Total Phenol content Determination:

800mu l of F.C reagent mixture, 2ml of 7.5% sodium carbonate was added to 200mu l of tge sample extract then the total content is diluted to 7 volumes with distilled water and the test tubes were kept in dark for incubation of 2hrs. The measuring absorbance used is 765nm. The standard solutions used is 0 to 0.5 concentrations of Gallic acid dilutions. The Gallic acid in mg/ml of extract determined the presence of phenols (Ramamoorthy et al., 2007).

Total Protein content determination:

Bradfords method is used to determine the total protein content. 3ml of Bradfords reagent is added to the 100mu l of the sample extract and incubate in dark for 5mins. The absorbance was read at 595nm. 0.1mg/ml to 0.5mg/ml Bovine serum albumin dilutions are used as standard dilutions.

Determination of saponins

20ml of 20% ethanol was used to disperse 2g of each sample. The suspension is heated in the hot water bath for 4h at about 55oc with continuous stirring. The mixture is filtered and 20ml of 20%ethanol is used to re-extract the residue. The water bath at about 90°c was employed to reduce the combined extracts to 40ml. To the 250ml separatory funnel, the concentrate was transferred and 2ml of diethyl ether was added and shaken vigorously. The purification process is repeated after recovering the aqueous layer.6ml of n-butanol was added and the combined n-butanol extracts were washed twice by using 1ml of 5% aqueous sodium chloride. The water bath was used to heat the remaining solution. The

samples were dried in the oven to a constant weight by the process of evaporation (Obadoni & Ochuko, 2001).

Total Alkaloid content determination:

To 1g of a powdered sample, 40ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. The filtrate is concentrated to $1/4^{th}$ of its original volume by using the water bath. The concentrated ammonium hydroxide is added dropwise to the extract until the precipitation is completed. The solution is allowed to settle. The dilute ammonium hydroxide is used to wash the collected precipitate and then filtered. The residue was dried and weighed (Gracellin et al., 2013).

Total Flavonoids Content Estimation:

5ml of 2% $AlCl_3$ prepared in methanol was mixed with the same volume and mixed with the same volume of extract solution. After 10 min, absorbance was taken at 415nm against blank. Blank was prepared as 5 ml of extract mixed with 5 ml of methanol without AlC_3. Catechin was used to prepare a standard graph (Ramamoorthy & Bono, 2007).

Total Carbohydrate determination:

To the 1ml of the sample solution, 1ml of 5% phenol and 5ml of concentrated H_2SO_4 was added and the absorbance was read at 488nm after 10mins against blank for estimating the polysaccharide content. The standard solution of glucose is used to compare. The blank is prepared by adding 1ml of distilled water to 1ml of 5% phenol and 5ml of concentrated H_2SO_4.

RESULTS AND DISCUSSION

TANNINS:

Many species of plants consists of polyphenolic biomolecules such as Tannins which play a important role in protecting from predation and regulates plant growth (Katie E. Ferrell et al., 2006). Martin and Synge discovered the paper chromatography methods for the first time for determining the phenolic constituents a highest tannins content was found in *Ocimum basillicum* and *Ocimum sanctum* (green) as 0.2273 and 0.2170mg/ml whereas *Ocimum gratissimum, Ocimum kilimand scharicum* shown the least concentration of 0.1950mg/ml, 0.1910mg/ml respectively. The leaf, bud, seed, root and stem tissues such as secondary phloem and xylem contains Tannins which regulate the growth of these tissues.

SAPONINS:

Saponins contains nitrogen-free glycosides such as a sapogenin and a sugar. The sapogenin may be a steroid or a triterpene and the sugar moiety is glucose, galactose, pentose or methyl pentose (Stecher et al., 1960). The marine organisms such as sea cucumber is used to isolate saponins (Hostettmann & Marston, 1995; Riguera, Ricardo 1997). As a soap, the root of the soapwort plant was used. ("saponins, Cornell uni, 2008). The unripe fruit of Manilkara zapota contains Saponins resulting in highly astringent properties. Saponins are present in the various parts of the plant leaves, stems, roots, bulbs, blossom, and fruit. Quil A, an extract from Quillaja Saponaria Molina (Hostettmann & Marston, 1995) used as adjuvants in development of vaccines (Sun et al., 2009). In animal feeding, Saponins are used for their effects in ammonia emission (USNND, 2010). In our study Saponins are found in a higher concentration of 20mg/ml in Ocimum basillicum and Ocimum sanctum purple compared to others.

PHENOLS:

They are the organic compounds consisting of Hydroxyl (OH) group attached to a carbon atom that is part of an aromatic ring . They are used as intermediates in household products and for industrial synthesis. They are used in mouth wash and household cleaners. They are the first surgical antiseptic used. Phenols are used in the manufacture of plastics, explosives such as picric acid, drugs such as aspirin in the industry as a raw material. The essential oils of plants consists of complex phenols which are used as flavourings and aromas. The vanilla beans is used for the extraction of Vanillin in vanilla as the principle flavouring agent. The wintergreen is used for the isolation of Methyl salicylate which has a characteristic minty taste and odor. In this study, the phenol

content of Ocimum species sample extracts was in the range of 0.32 to 0.355mg/ml.

ALKALOIDS:

They are naturally occurring organic compounds containing basic nitrogen atoms. The organisms like bacteria, fungi, plants and animals are known to produce phenols. The crude extract of these organisms by acid base extraction is used to purify the phenols. It exhibits medicinal properties including antimalarial (eg quinine), antiasthma (eg ephedrine), anticancer (eg homoharringtonine) (Kittakoop et al., 2014) analgesic (eg morphine) (Raymond et al., 2010). antihyperglycemic activities (eg piperine) (Qiu et al., 2014). They dissolve readily in organic solvents such as diethyl ether, chloroform, Caffeine (2013). Cocaine (2013). Nicotine (2013), Morphine (2013). but dissolve very poor in water. Most alkaloids have a bitter taste when ingested.

The alkaloids have been produced in plants after being gazed by herbivorous animals. Few of them have the potential to crash the alkaloids (Caffeine 2013). The serious facial deformations in lambs born by sheep after grazing has been caused by the alkaloid produced in the leaves of corn lily . The compound responsible for deformities are cyclopamine (Fattorusso). In the present study, Ocimum basillicum and Ocimum kilimandscharicum and Ocimum sanctum (green) have higher concentration i.e is 20mg/g compared to others.

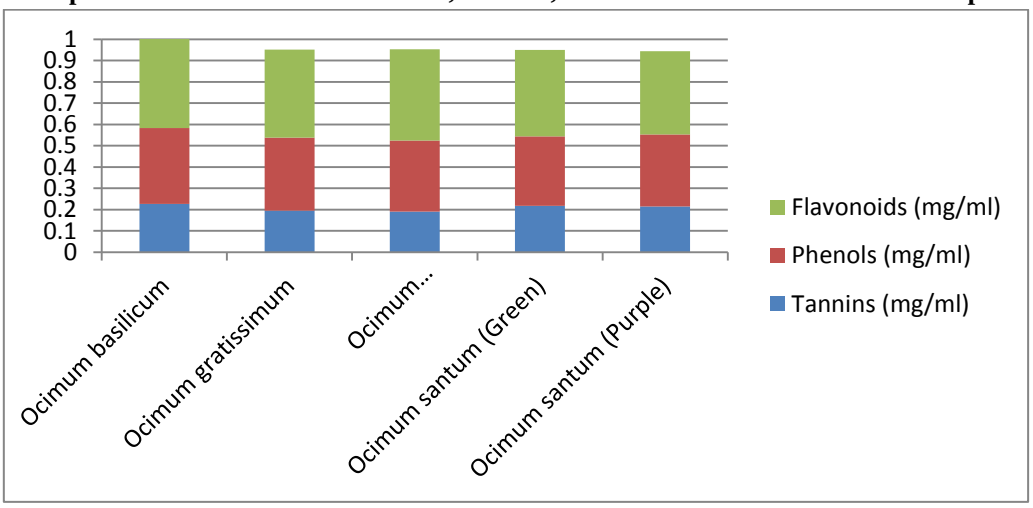
FLAVONOIDS:

The fruits, vegetables and, plant-derived beverages consists of naturally occurring polyphenolic compounds known as Flavonoids. The antioxidant effects associated with various diseases such as cancer, Alzheimer's disease, atherosclerosis are present in flavonoids (Lee et al., 2009; Ovando et al., 2009; Burak & Imen, 1999). The attractive colors of flowers, fruits, and leaves are due the presence of flavonoids. The protection against ultraviolet radiation, pathogens, herbivores are provided by flavonoids. The antibacterial, antiviral, anti-inflammatory properties are exhibited by flavonoids. The cardiovascular disease is inversely correlated with the mortality rate due to the presence of flavonoids. A great variety of fruits and vegetables, including tea, coffee, and other grains consists of flavonoids like Quercetin and rutin. It is reported that flavonoids have a preventive effect on cancer, anti-inflammatory, antiviral activities. In the present study, *Ocimum basillicum* showed the highest flavonoid content is 0.4373mg/ml. As compared to other constituents, Alkaloids and saponins are the greater constituents present in *Ocimum basillicum, Ocimum kilimandscharicum* and *Ocimum sanctum* (purple).

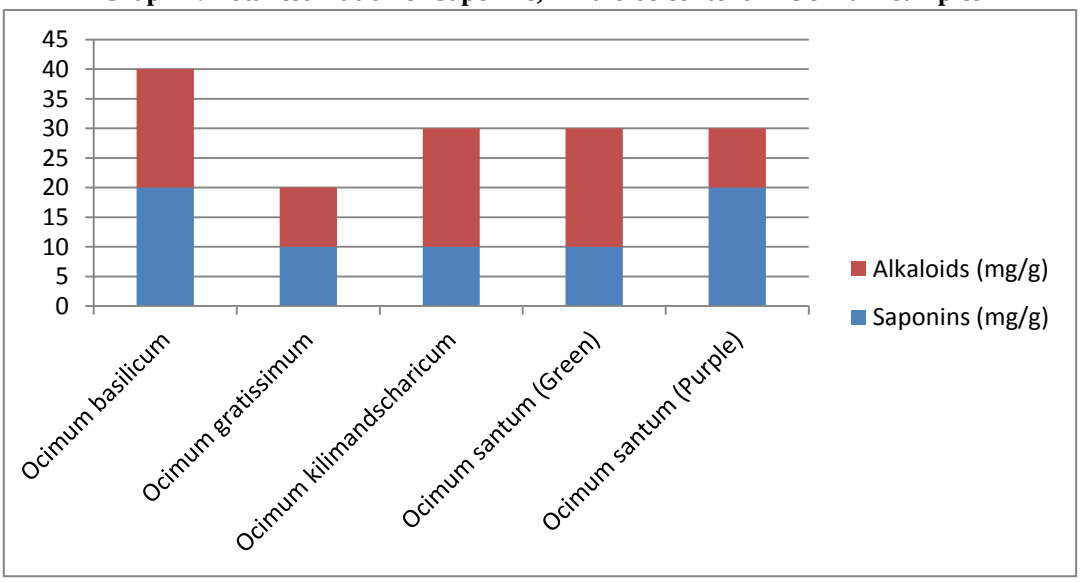
In the below table *Ocimum basillicum* has shown the higher concentration of 0.2273mg/ml of tannins, 20mg/g of saponins, 0.4373mg/ml of flavonoids, whereas *Ocimum gratissimum*, *Ocimum kilimandscharicum* shown the least concentration of 0.1950mg/ml, 0.1910mg/ml of tannins, 10mg of saponins, 0.4137mg/ml and 0.4282mg/ml of flavonoids. Therefore *Ocimum basillicum* showed a higher concentration of tannins, saponins, and flavonoids compared to other species of Ocimum. In other research studies, it has been reported that age is one of the factors to determine the phytochemical contents. The different chemical constituents are present in same species of plants grown in different geographical conditions has been studied. The phytochemicals are produced as the end product of plant metabolism for fighting against pathogens. Phytochemical can also be toxic apart from its uses for medicinal purposes in the human body (Trease & Evans, 1989). Saponins have positive effects on the cholesterol levels of blood, to combat cancer, enhancement of the immune system (Sale & Maji, 2006).

The steroid drugs, Corticosteroids, the contraceptives, stimulants of sex hormone have been synthesised by using phytochemicals (Aliyu et al., 2008; USNND, 2010). Tannins are the polyphenols which have properties like binding, precipitating the proteins and organic compounds. They are known to heal wounds along with the properties of fighting against viral diseases, cancer and inflammation (Sale & Maji, 2006).

Graph 1: Total estimation of Tannins, Phenols, Flavonoids content in Ocimum samples



Graph 2: Total estimation of Saponins, Alkaloids content in Ocimum samples



CONCLUSION

The analysis of phytochemicals in the present study has proved that in all the Ocimum species the presence of the phytochemicals which are known as biologically active compounds such as phenols, flavonoids, saponins, tannins, alkaloids has antioxidant activity. A higher concentration of phytochemicals is found in *Ocimum basillicum* especially flavonoids. The constituents of Ocimum plants have shown a greater advantage in the treatment of various diseases. The phytochemicals present in all the sources of Ocimum has shown higher antioxidant activity. Therefore from our present work, it can be concluded that Phytochemical components are the rich sources of antioxidants which has a more beneficial role in the pharmacology.

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