Original Research Article **Open Access**

SCREENING OF ANTIBACTERIAL POTENTIAL AND ANTIOXIDANT ACTIVITIES OF BASIL LEAVES *(OCIMUM BASILICUM.L)* FROM DIFFERENT ECOTYPES OF TAMILNADU.

Murali M and Prabakaran G[1]

PG and Research Department of Botany, Government Arts College, Dharmapuri-635 705, Tamilnadu, India.

Abstract

Ocimum basilicum.L (sweet basil) are used traditionally medicine as antispasmodic, aromatic and carminative agents whose leaves exhibit more antibacterial and antioxidant properties against infectious diseases. In this study leaves of *O.basilicum* collected from various ecotypes and each sample extracted with different solvents like methanol, petroleum ether, Acetone, Chloroform and Aqueous. Following extracts were examined the effect of antibacterial and antioxidant-DPPH scavenging and Catalase activities all the extracts and samples are significant properties. Results showed that highest antibacterial activity in methanol extract in courtallam sample against *E.coli*(24mm) and *S.aureus*(25mm) in acetone and methanolic extracts. The highest DPPH scavenging radicals activities (82.59± 0.133) in methanolic extracts of courtallam sample and catalase antioxidant activities(0.096±0.005 units mg/protein) maximum in methanol extracts of courtallam sample. Our findings identified appropriate solvent for extracting active principle compounds for antibacterial and antioxidants.

Keywords *Ocimum basilicum*, DPPH, Antioxidant and Antibacterial activity

INTRODUCTION

Ocimum basilicum L. commonly called as Sweet Basil, is a popular annual aromatic and medicinal plant belongs to family Lamiaceae is native plant of Indo-Malayan region. The genus *Ocimum* consists of 30-160 species, most important species being utilized as a source of essential oil [1]. The plant grows 20-50 cm high, erect and sometimes bushy. The leaves are ovate or oblong, very lightly toothed, shinny with deep vein marking. The flowers are white, labiate are six in blossom, pedicled, almost sessile, axillary, false whorls. Calyx is bilabiate and the corolla is 4-lobed [2].

Ocimum basilicum which contains plenty of phytoconsitituents with significant nutritional as well as antioxidant capabilities and health benefits [3]. It is used an aroma to improve the taste, smell and digestibility of food and ingredient in various dishes and food preparations, especially in the Mediterranean cuisine. Leaves and flowering parts of *O.basilicum* are traditionally used as treatment of headaches, coughs, diarrhea, constipation, warts, worms and kidney malfunction [4]. antispasmodic, aromatic, carminative, digestive, alactogogue, stomachic, and tonic agents.

In addition to it has been extensively utilized in food as a flavoring agent, perfumery and medical industries. The leaves and flowering tops of the plant are perceived as carminative, galactogogue, stomachic and antispasmodic in folk medicine. The *O. basilicum* essential oils exhibited a wide and varying array of chemical compounds, depending on variations in chemo types, leaf and flower colors, aroma and origin of the plants. The chief constituents include chavicol methyl ether or Estragole, linalool and Eugenol [5].

Plant extracts have been recognized as a future source of new antimicrobial which are being derived from microorganisms and possess

Address Correspondence @ PG and Research Department of Botany, Government arts College, Dharmapuri 635 705, Tamil Nadu, India.
Email: gpbiotek@gmail.com

active ingredients to cure disease or relive pain [6]. The bioactive compounds produced by plants either acts interfering with the metabolism of microbes infecting them as pathogenic or symbiotic. In recent years multiple drug resistance has developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases. Resistance to antibiotics is recognized at present as a major global public health problem. The number of resistance strains of pathogens is growing at a alarming rate since penicillin resistance and multi resistance *pneumococci* caused a major problem throughout world [7].

Antioxidant are our first line of defense against free radical damage and critical for maintaining optimum health and wellbeing. Free radicals are chemical species which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Free radicals are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function [8]. Free radicals can initiate the oxidation of biomolecules, such as protein, lipid, amino acids and DNA which will lead to cell injury and can induce numerous diseases. Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases [9].

MATERIALS AND METHODS

Collection of Plants

Healthy and Fresh leaves of *Ocimum basilicum*.L (Sweet basil) were collected from the different habitates (ecotypes) of Tamilnadu in Ramanathapuram, Courtallam, Chidambaram, Valparai and Yercaud. The plant sample was identified by renowned taxonomist and authenticated in Botanical Survey of India (BSI) Coimbatore, Tamilnadu Southern India. Voucher number BSI/SRC/5/23/2013-14/TECH/1287.

Plant extraction

The collected various habitat leaf material were dried under shade until all the water molecules evaporated. After drying, the plant materials were ground well using mechanical blender fine powdered of five sample leaves was extracted with using solvents Methanol, Acetone,Petroleum ether, chloroform and Aqueous were successively for extraction of active principles from the dried and pulverized plant using soxhlet extraction method. The extraction was continued for 72 hrs, till the extraction became colorless. The extracts were concentrated on vacuum rotary evaporator and their percentage yields were calculated and labeling for further uses.

Antibacterial activity

Antibacterial activity was evaluated by well diffusion method on using MHA medium. The sterile medium (20 ml) was poured into Petri plates and allowed to cool in a sterile condition and plates were inoculated with $1x10^5$ cfu culture of Gram- positive *Staphylococcus aureus* and gram- negative *Escherichia coli* test bacteria. The concentration of bacterial cells in the suspension was adjusted to minimum of $1x10^5$ cfu/ml in Muller Hinton broth solution. Agar well of 6mm diameter were made in the plates. The desired five different samples of different concentrations (5mg, 10mg, 20mg and 40mg/ml) of the Methanol, Acetone, Petroleum ether, Chloroform and Aqueous extracts were added to each well into MHA plates already seeded with the standardized inoculums (5×10^5) of the test bacterial cells. The control antibiotic is used as ciproflaxin. All test plates were incubated at 37°C for 24hrs.

Antioxidant activity

Determination of antioxidant activity from *Ocimum basilicum*

DPPH Radical Scavenging Activity [10]

The free radical scavenging activity of the plant extracts was determined by 1, 1- diphenyl-2-picrylhydrazyl (DPPH). 1ml of 0.1mM methanolic DPPH solution was added to 3ml of different

Table 1: Antibacterial activity of different extracts of *Ocimum basilicum*.L leaves from various habitats against the bacterial strains on well diffusion method.

Sample	Extract	*E.coli*					*S.aureus*				
	SOLVENTS	5mg	10mg	20mg	40mg	AB	5mg	10mg	20mg	40mg	AB
Valparai	Acetone	-	-	11	14	-	-	-	10	10	15
	Methanol	-	-	12	15	9	-	-	-	11	11
	Chloroform	-	-	-	-	-	-	-	11	11	13
	Water	-	-	-	12	-	-	-	-	-	20
	Petroleum ether	-	-	-	-	11	-	-	-	-	11
Chidambaram	Acetone	-	-	10	12		-	-	9	10	14
	Methanol	10	11	14	14	9	-	-	-	10	11
	Chloroform	-	-	-	10	11	-	-	10	11	13
	Water	-	-	-	-	-	-	-	-	-	21
	Petroleum ether	-	-	-	-	11	-	-	-	-	11
Yercaud	Acetone	22	24	28	32	-	13	15	18	20	12
	Methanol	20	23	26	29	9	12	15	17	19	11
	Chloroform	-	-	-	-	11	12	14	16	18	10
	Water	23	27	28	30	-	11	14	18	18	19
	Petroleum ether	22	-	-	11	11	-	-	-	12	11
Courtallam	Acetone	11	27	31	32	-	-	14	17	18	15
	Methanol	24	28	30	32	9	16	18	20	20	11
	Chloroform	23	30	31	36	-	15	20	20	22	13
	Water	23	26	30	32	10	10	16	18	20	20
	Petroleum ether	-	-	-	12	11	-	-	-	-	11
Ramanathapuram	Acetone	13	18	20	24	-	25	28	29	31	15
	Methanol	12	16	17	20	9	25	31	32	33	11
	Chloroform	12	15	17	20	-	23	26	29	32	13
	Water	15	17	18	20	-	23	26	29	30	20
	Petroleum ether	-	-	17	20	12	-	-	-	-	11

concentrated essential oil (0.5, 1.0, 1.5, 2.0 and 2.5mg/ml) of Methanol, Acetone, Petroleum ether, Chloroform and Aqueous extracts with all the five samples of *O. basilicum*. The mixture was vigorously shaken and left to stand for 30 minutes under subdued light. The absorbance was measured at 517 nm in a UV spectrophotometer. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid, which is a good antioxidant, was taken as a standard in this study. All the measurements were conducted in triplicate and statistical analysis used by performing mean± and standard deviation. . The DPPH radical scavenging activity was calculated as percentage inhibition by using the following equation:

DPPH Scavenging activity (%) = (1-As/Ac) ×100mg

Enzymatic method *(*Catalase activity)

The antioxidant activity Enzymatic method Catalase activity was followed by Sinha *et al.,*1972 method [11] in which the reaction mixture (1.5ml vol) contained 1.0ml of 0.01M phosphate buffer (pH7.0),0.1ml of different concentrated plant extracts and 0.4ml of 2M H_2O_2.The reaction was stopped by the addition of 2.0ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio).

Table 2: DPPH -scavenging antioxidant activities of different extracts of *Ocimum basilicum* .(L) leaves from various habitats

SAMPLES	concentration of samples mg/ml	Different solvent extracts				
		Acetone	Chloroform	Petroleum ether	Aqueous	Methanol
VALPARAI	0.5	75.84±0.364	71.20±0.192	17.84±0.149	55.33±0.270	71.56±0.471
	1.0	78.64±0.363	75.69±1.752	21.78±0.135	55.71±0.392	78.49±0.458
	1.5	82.38±0.230	74.35±0.184	36.44±0.470	65.62±0.178	81.41±0.172
	2.0	84.55±0.340	77.15±0.081	40.57±0.192	68.50±0.440	84.37±0.176
	2.5	86.63±0.281	80.62±0.374	42.33±0.220	71.25±0.165	86.89±0.073
CHIDAMBARAM	0.5	34.46±0.421	21.58±0.173	37.65±0.110	74.32±0.110	69.40±0.385
	1.0	50.26±0.278	28.25±0.161	55.34±0.220	79.75±0.210	71.84±0.743
	1.5	71.35±0.110	31.54±0.110	64.15±0.052	82.25±0.263	75.66±0.210
	2.0	78.56±0.245	37.54±0.105	71.76±0.106	84.44±0.111	78.10±0.094
	2.5	79.66±0.095	40.04±0.121	80.09±0.100	87.85±0.075	80.04±0.030
YERCAUD	0.5	56.21±0.100	19.43±0.212	27.47±0.231	61.47±0.130	72.07±0.075
	1.0	62.22±0.104	21.74±0.175	30.35±0.134	64.47±0.387	74.75±0.485
	1.5	66.64±0.105	35.25±0.308	38.74±0.234	71.33±0.290	83.58±0.223
	2.0	72.42±0.259	39.63±0.170	45.84±0.166	81.59±0.072	85.41±0.346
	2.5	77.21±0.105	46.72±0.170	56.79±0.153	87.44±0.232	88.60±0.223
COURTALLAM	0.5	75.60±0.204	79.49±0.315	33.39±0.162	79.65±0.254	82.59±0.127
	1.0	80.79±0.110	84.15±0.115	41.25±0.020	82.63±0.162	84.29±0.133
	1.5	83.47±0.140	82.77±0.283	50.06±0.050	84.28±0.075	85.68±0.291
	2.0	86.61±0.179	85.13±0.050	59.25±0.072	87.62±0.079	87.60±0.361
	2.5	89.48±0.220	88.71±0.144	63.41±0.320	89.10±0.095	93.57±0.361
RAMANATHAPURAM	0.5	64.78±0.120	4.68±0.300	30.21±0.075	28.70±0.225	75.55±0.208
	1.0	74.57±0.195	16.09±0.032	39.22±0.125	42.22±0.472	83.37±0.382
	1.5	83.54±0.205	20.22±0.556	45.66±0.293	50.11±0.061	87.69±0.250
	2.0	86.75±0.085	28.14±0.070	51.64±0.250	59.63±0.340	89.46±0.265
	2.5	89.52±0.151	29.63±0.278	58.58±0.313	74.51±0.446	90.00±0.026

Values are means ± standard deviation at p≤0.05

Then the absorbance was noted at 620nm. The same procedure was followed for all the five different sample of *O.basilicum* with different concentration of Acetone, Petroleum ether, Chloroform, Aqueous and Methanol (0.5, 1.0, 1.5, 2.0 and 2.5mg/ml) extracts. The Catalase activity was expressed as µMol of H_2O_2 consumed/min/mg protein.

RESULTS AND DISCUSSION

The antibacterial activity well known conventional method used for this study is disc diffusion agar. The results of antibacterial activity were done for Acetone, Methanol, Chloroform, Petroleum ether and Aqueous extracts of different ecotype *Ocimum basilicum* samples. Evaluation of variance that all the five samples and dilutions of different extracts of basil essential oils have significant antibacterial activity against the bacterial strains In least concentration (5mg/ml) of various extracts of *Ocimum basilicum* leaves have good response against test organisms. The minimum concentration of methanolic extract of *O.basilicum* leaf sample showed maximum zone of inhibition against the

Table 3: Catalase antioxidant activities of different extracts of *Ocimum basilicum* .(L) leaves from various habitats

SAMPLES	Concentration of samples mg/ml	Different solvent extracts				
		Acetone	Chloroform	Petroleum ether	Aqueous	Methanol
VALPARAI	0.5	0.010±0.001	0.021±0.001	0.011±0.001	0.022±0.001	0.022±0.001
	1.0	0.021±0.001	0.032±0.002	0.034±0.002	0.043±0.002	0.037±0.001
	1.5	0.041±0.001	0.052±0.001	0.054±0.002	0.059±0.017	0.055±0.019
	2.0	0.060±0.001	0.058±0.003	0.060±0.001	0.085±0.003	0.086±0.014
	2.5	0.070±0.001	0.076±0.002	0.088±0.001	0.395±0.514	0.12±0.001
CHIDAMBARAM	0.5	0.041±0.001	0.011±0.002	0.012±0.001	0.011±0.001	0.008±0.007
	1.0	0.053±0.004	0.022±0.001	0.055±0.001	0.023±0.001	0.023±0.002
	1.5	0.074±0.002	0.042±0.001	0.075±0.003	0.033±0.002	0.046±0.003
	2.0	0.083±0.003	0.052±0.001	0.084±0.003	0.055±0.014	0.056±0.002
	2.5	0.092±0.003	0.074±0.001	0.146±0.020	0.075±0.003	0.084±0.004
YERCAUD	0.5	0.010±0.001	0.023±0.003	0.032±0.001	0.052±0.002	0.059±0.001
	1.0	0.012±0.001	0.044±0.004	0.055±0.004	0.064±0.002	0.084±0.002
	1.5	0.035±0.001	0.055±0.004	0.072±0.002	0.083±0.003	0.175V0.003
	2.0	0.047±0.006	0.075±0.003	0.095±0.002	0.146±0.040	0.261±0.010
	2.5	0.075±0.002	0.085±0.003	0.125±0.002	0.135±0.003	0.333±0.002
COURTALLAM	0.5	-	-	0.012±0.001	0.023±0.001	0.093±0.001
	1.0	0.023±0.005	0.012±0.001	0.032±0.001	0.052±0.001	0.173±0.005
	1.5	0.043±0.002	0.044±0.002	0.053±0.002	0.074±0.001	0.283±0.014
	2.0	0.063±0.001	0.062±0.002	0.075±0.002	0.093±0.002	0.383±0.005
	2.5	0.084±0.002	0.096±0.002	0.092±0.000	0.126±0.005	0.523±0.005
RAMANATHAPURAM	0.5	0.012±0.001	0.032±0.001	0.021±0.001	-	0.054±0.001
	1.0	0.032±0.001	0.055±0.003	0.032±0.001	0.012±0.001	0.142±0.001
	1.5	0.045±0.003	0.072±0.001	0.055±0.002	0.033±0.001	0.224±0.003
	2.0	0.052±0.001	0.095±0.002	0.086±0.002	0.054±0.001	0.217±0.161
	2.5	0.072±0.001	0.134±0.002	0.111±0.001	0.064±0.002	0.416±0.003

Values are means ± standard deviation at p≤0.05

pathogen *E.coli* at the concentration of 5mg/ml(24mm) in courtallam compare to Yercaud(20mm) and Ramanathapuram(12mm)at same extract followed by Aqueous extract also significantly showed maximum inhibition against *E.coli* in Courtallam and Yercaud (23mm) compare to acetone(22mm) extract. minimum concentration (0.5mg/ml) of all the extracts exhibits no inhibition against *E.coli* in Valparai and Chidambaram Samples. Minimum concentration (5mg/ml) of Acetone and Methanol extracts of Ramanathapuram (26mm and 25mm) sample of *O.basilicum* has showed maximum inhibition zone against *S.aureus* followed by same sample of Aqueous extracts(23mm)compare to courtallam at methanol extract(16mm). Similarly lowest concentration of all the extracts of Valparai and Chidambaram *O.basilicum* leaf sample no antibacterial activity was observed (Table 1).

The results of Antioxidant activity were also observed in different natural habitats of *Ocimum basilicm* leaves in various extracts the antioxidant activity is present in both the enzymatic and non

enzymatic methods. The method of DPPH assay the effective concentrations of the essential oil required to scavenge DPPH radical and the scavenging values as inhibition percentage at various concentrations. Ascorbic acid was used as standard control and the antioxidant capacity of the five samples to radical scavenging activity in five extracts, the least concentration (0.5mg/ml) of methanol extract exhibited a dose dependent increase with radical scavenging activity of (82.59±0.133) was showed in Courtallam, followed by Yercaud (72.07±0.075), Valparai (71.56±0.471) and Chidambaram (69.40±0.385) in methanol extract, which is lower than the DPPH scavenging activity in Ramanathapuram (4.38±0.16) in chloroform extract.(Table-2)

Among the five samples of *O.basilicum* in different extractions of least concentration (0.5mg/ml) the methanol extract more efficiently the highest catalytic activity was observed in Courtallam sample (0.096±0.005mg protein) and lowest in same extract of Valparai (0.011±0.001mg protein) followed by yercaud (0.051±0.007), Ramanathapuram (0.0466±0.005 units mg/protein), Chidambaram (0.013±0.005 unit mg/protein) in same extract (table-3). Catalase is a heme protein which catalyses the reduction of hydrogen peroxides and protects the tissues from hydroxyl radicals. Therefore reduction in the activity of this enzyme (CAT) may result in a number of deleterious effects due to the accumulation of superoxide anion and hydrogen peroxide [12].

The results of our study showed the better activity of essential oil of *O.basilicum* on Gram – positive and gram negative germs. Many literature including difference of methodology of evaluating the antibacterial properties and compositions from different geographical regions makes its difficult and even impossible comparing the studies on the antibacterial activities of *O.basilicum* [5]. These results showed that the essential oil to which ocimum basilicum play an important role as antibacterial and antioxidants, the high amount of polyphenols is used in therapy [13]. The methanol extract of the leaves of *leucas aspera* the determination of antioxidant capacity by reducing assay, found that reducing power was increase when increasing in concentration of crude extracts [14].The crude Ethyl acetate, methanol and water extracts were evaluation of antioxidant activity in several Chinese herbs performed by DPPH assay compared to L-ascorbic acid all the extracts showed greater activity than that of the standard[15].Excessive generation of Reactive Oxygen Species(ROS) leads to a variety of pathological process such as inflammation , diabetes, hepatic damage and cancer [16]. Antioxidants are secondary metabolites and their contents in plants depend on varied stress conditions of vegetations [17].

CONCLUSION

The results of the present study suggest that basil plant *Ocimum basilicum* collected from different localities of Tamilnadu at Southern India significantly difference Antibacterial and Antioxidant activity. On the basis of antibacterial activity methanol extracts showed maximum zone of inhibition against the tested organisms. Antioxidant potential for all samples showed highest effect of DPPH and catalase activity in methanol crude extracts followed by acetone in courtallam sample. The antibacterial and antioxidant difference which can be attributed to different growth conditions, genetic factors, geographical variations and analytical procedures. However, further investigation to establish how the components interact to provide antioxidant activity studies should also be extended to evaluating the practical effectiveness of plant material can be related to antidiuretic and anti inflammatory properties.

REFERENCES

[1] Talaat IM and Balbaa LK (2010) Physiological response of sweet basil plants (Ocimum basilicum L.) to putrescine and trans-cinnamic acid. American-Eurasian Journal of Agricultural and Environmental Science. 8(4):438-45.

[2] Patil DD, Mhaske DK and Wadhawa GC (2011) Antibacterial and Antioxidant study of Ocimum

basilicum Labiatae (sweet basil). Journal of Advanced Pharmacy Education & Research. 2:104-12.

[3] Shafique M, Khan SJ and Khan NH (2011) Study of antioxidant and antimicrobial activity of sweet basil (Ocimum basilicum) essential oil. Pharmacologyonline. 1:105-11..

[4] Lee SJ, Umano K, Shibamoto T and Lee KG (2005) Identification of volatile components in basil (Ocimum basilicum L.) and thyme leaves (Thymus vulgaris L.) and their antioxidant properties. Food Chemistry. 91(1):131-7.

[5] Moghaddam AM, Shayegh J, Mikaili P and Sharaf JD (2011) Antimicrobial activity of essential oil extract of Ocimum basilicum L. leaves on a variety of pathogenic bacteria. Journal of Medicinal Plants Research. 5(15):3453-6..

[6] Okigbo RN, Eme UE and Ogbogu S (2008) Biodiversity and conservation of medicinal and aromatic plants in Africa. Biotechnol. Mol. Biol. Rev. 3(6):127-134.

[7] Cohen FL and Tartasky D (1997) Microbial resistance to drug therapy: a review. American Journal of Infection Control. 25(1):51-64.

[8] Gulcin I (2005) The antioxidant and radical scavenging activities of black pepper seeds. International journal of food science nutrition, 56(7):491-499.

[9] Soni A and Sosa S (2013) Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. Journal of Pharmacognosy and Phytochemistry.1;2(4).

[10] Dhal YO, Deo BA and Sahu RK (2012) Antioxidant activity of enzymatic extracts of Curcuma zedoaria (Christm.). Int. J. Pharm. Pharmaceut. Sci. 4:343-346.

[11] Sinha AK (1972) Colorimetric assay of catalase. Analytical biochemistry, 47(2):389-394.

[12] Sajeeth CI, Manna PK and Manavalan R (2011) Antioxidant activity of polyherbal formulation on streptozotocin induced diabetes in experimental animals. Der Pharmacia Sinica. 2(2):220-226.

[13] Benedec D, Vlase L, Hanganu D and Oniga I (2012) Antioxidant potential and polyphenolic content of Romanian Ocimum basilicum. Digest Journal of Nanomaterials and Biostructures. 7(3):1263-70.

[14] Ali MS, Nabi MM and Rahman MAA (2013) *In-vitro* antioxidant and cytotoxic activities of methanol extract of *Leucas aspera* leaves. Journal of Pharmacognosy and Phytochemistry, 2(1): 8-13.

[15] Jayanthi P and Lalitha P (2011) Reducing power of the solvent extracts of Eichhornia crassipes (Mart.) Solms. International Journal of Pharmacy and Pharmaceutical Sciences. 3(3):126-8.

[16] Ilavarasan R, Mallika M and Venkataraman S (2006) Anti-inflammatory and free radical scavenging activity of Ricinus communis root extract. Journal of Ethnopharmacology, 103(3):478-80.

[17] Buřičová L and Reblova Z (2008) Czech medicinal plants as possible sources of antioxidants. Czech J Food Sci. 26(2):132-8.

Citation: Murali M and Prabakaran G (2017) Screening of antibacterial potential and antioxidant activities of basil leaves (*Ocimum basilicum*. L) from different ecotypes of tamilnadu. Int J Adv Interdis Res, 4 (4): 17-23 .

Received: Nov 03, 2017 **| Revised:** Dec 07, 2017 **| Accepted:** Dec 08, 2017 **| Published:** Dec 26, 2017