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PHYTOCHEMICAL ANALYSIS, ANTI-MICROBIAL AND ANTI-OXIDANT ACTIVITY OF *OCIMUM BASILICUM*

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ABSTRACT

Ocimum basilicum Linn (Lamiaceae) is familiar as a plant having healing properties and has received consent to be used for variety of ailments in many countries The aqueous soxhlet extract which is ordinarily utilized in folkloric drug, while methanol and hexane soxhlet concentrates were exposed to phytochemical screening, the phytochemical analysis of the aqueous extract revealed that steroids, glycosides, carbohydrates, proteins, alkaloids, oils, terpenoids are present. The phytochemical investigation of other two fractions contains glycosides, carbohydrates, proteins, alkaloids, oils and terpenoids. In addition, hexane extract contains reducing sugar and tannin. *O. basilicum* were found to possess antioxidant and In vitro antibiotic activity against *E. Coli*, *Staphylococcus aureus, Klebsiella pneumonia, Bacillus sps, Aspergillus Niger* and Candida *albicans* at high doses, in conclusion, this study has shown that *O. basilicum* contain primary and secondary metabolites that can be pharmacologically useful as well as posses some antibacterial, antifungal and antioxidant properties.

KEYWORDS: Ocimum basilicum Linn, *RbcL* Molecular markers, DNA Barcoding, Phytochemical screening, Antibiotic activity.

INTRODUCTION

The Genus *Ocimum* holds in excess of 150 species, for the most part called as 'Basil. (Paton, *et al* 1999). *Ocimum basilicum L*., Known as basic basil, is a yearly fragrant herb having a place with the Lamiaceae family. (Prakash, 1990). It is cultivated all around the globe. it otherwise called as Sweet basil and classified as "lord of herbs" ((Mohammad an *et al) Ocimum basilicum L* is the most monetary species to develop. (Marotti *et al.*, 1996). Dried leaves of basil are utilized to season numerous items in the nourishment business. Its basic oil is utilized in the scent and cologne, beautifying agents, wellbeing, pharmaceutical and sustenance ventures (Harisaranraj., 2008). In light of Khosla's (1995) reports, the focal point of basil assorted variety is in the tropical and subtropical districts of Africa, Asia and South America. In sweet basil antimicrobial specialists are available normally because of which they show antimicrobial impacts against different pathogenic microorganisms (Ozcan and Mohammad An *et al* 2002 and 2011).

The present examination includes the gathering of *Ocimum basilicum L* leaves from Palakarai zone, Tiruchirappalli, Phytochemical examination was completed by various solvents at that point screen the impact of *Ocimum basilicum L* on differentiated pathogenic microorganisms such as *Staphylococcus aureus, Bacillus sps, E. coli, Klebsiella sps, Candida albicans, Aspergillus niger*. The microorganisms were picked in this investigation are universal in our condition and are the essential causative operators of most regular contaminations and In Vitro Scavenging activity also carried out by various solvents of *O. basilicum L.*

MATERIALS AND METHODS

Plant Collection and Identification

Fresh leaves of *O. basilicum* were collected from the Palakarai area, Tiruchirappalli, Tamil Nadu, India and taxonomic identification of the collected plant material was confirmed at Department of Botany, St. Joseph's College, Tiruchirappalli. The leaves were rinsed with double distilled water, followed by exposure to 0.1% mercuric chloride solution. The leaves were then shaded dried for a period of two weeks. The dried leaves were powdered and stored for further investigation.

Preparation of Extracts

Soxhlet extraction of the plant materials was carried out with three solvents namely hexane, methanol and water. 50 grams of the powdered plant material was packed in Whatman No.1 filter paper and were extracted separately with 300 ml of the solvents for 48 hours. The extracts

were then concentrated at room temperature and stored at 40ºC for further use.

Phytochemical Screening of the extracts
The chosen extracts were screened qualitatively for the presence of various bioactive compounds by employing a standard protocol devised by Evans *et al*., 1997.

Antimicrobial activity
Antibacterial activity
The antibacterial activity of the extracts was tested against four bacterial strains viz., *E. coli, Klebsiella sp, Staphylococcus aureus and Bacillus sp*. Standard well diffusion method (Kirby Bauer *et al*., 1966) employing Muller Hinton agar was performed and the plates were incubated at 37ºC for 24 hours.

Antifungal activity
The plant extracts were tested for their antifungal activity against two fungal strains viz., *Aspergillus niger* and *Candida albicans* on Potato Dextrose Agar plates. The plates were incubated at 37ºC for 48 hours.

Antioxidant activity
The ability of the extracts of *O. basilicum* to scavenge 1, 1- diphenyl-2 picrylhydrazyl (DPPH) was determined by the reported method (Alothman, et al., 2009). Ascorbic acid was used as standard and the solvent served as blank. Test samples were taken at different concentrations (100, 200, 300, 400 and 500µg/ml). DPPH reagent was added to the test tubes and the absorbance was determined at 517 nm.

Calculation
% Antioxidant activity = {(absorbance at blank) – (absorbance at test) / (absorbance at blank)} X 100.

RESULTS AND DISCUSSION

Collection of *Ocimum* species & Extract Preparation:
The plant material of *Ocimum basilicum* was collected from the Palakarai area, Tiruchirappalli, India. The plants were identified and the Herbarium sheet (No-2795) was deposited in Department of Botany, St. Joseph's College, Tiruchirappalli. The plant leaves were a shade dried and powdered. 50 gm powder sample was dis-solved in 300ml polar and non polar solvents like methanol hexane and distilled water and store it.

Figure 1: *Ocimum basilicum* Collection & Extract Preparation.

Phytochemical Screening
Phytochemical screening of plant extracts was done by the standard procedure devised by Evans *et al.*, 1997. The presence of compound indicates by + and absence of compound indicates by – symbols.

Table 1: Phytochemical screening of *O. basilicum*.

O. basilicum				
S. No.	**Experiments**	**Water**	**Methanol**	**Hexane**
1	Test for Carbohydrates: Molish's Test	+	+	+
2	Test for Glycosides :			
	a) Keller Killiani test	+	+	+
	b) Conc H2SO4 test	+	+	+
3	Test for Flavonoids			
	a) Lead acetate test	+	+	+
	b) Alkaline reagents test	+	+	+
4	Test for Reducing Sugar : Fehling's Test	+	-	+
5	Test for Steroids : Salkowski test	+	-	+
6	Test for Protein :Biuret Test	+	+	-
7	Test for Saponins : Glycosides Foam test	+	+	-
8	Test for Alkaloids : Mayer's test	+	+	+

9	Test for Phenol:Ferric chloride test	+	+	+
10	Test for Tannins			
	a) Lead acetate test	+	-	+
	b) Ferric chloride test	+	-	+
11	Test for Oils and fats			
	a) Spot test	+	+	+
12	Test for Terpenoids	-	-	+

Antimicrobial activity

Antibacterial activity

The antibacterial activity of the extracts was tested against four bacterial strains viz., *E.coli*, *Klebsiella sp*, *Staphylococcus aureus* and *Bacillus sp*. Standard well diffusion method (Kirby Bauer *et al*., 1966) employing Muller Hinton agar was performed and the plates were incubated at 37ºC for 24 hours. The Aqueous Extracts show high levels of zone of inhibition when compare to other solvents extract.

Table 2: Antibacterial activity *O. basilicum*.

Inhibition Zone diameter in mm (mean ±SD)								
Test Bacteria	**Hexane**		**Methanol**		**Aqueous**		**Positive Control**	
	Experimental	**Negative Control**	**Experimental**	**Negative Control**	**Experimental**	**Negative Control**	**Vancomycin**	**Gentamycin**
Gram - Positive								
E.Coli	*12*	*–*	*15*	*–*	*16*	*–*	*12*	*17*
K.Pneumoniae	*13*	*–*	*11*	*–*	*12*	*–*	*12*	*15*
Gram - Negative								
S. aureus	*13*	*–*	*15*	*–*	*16*	*–*	*20*	*23*
B. Subtilis	*0*	*–*	*9*	*–*	*12*	*–*	*25*	*30*

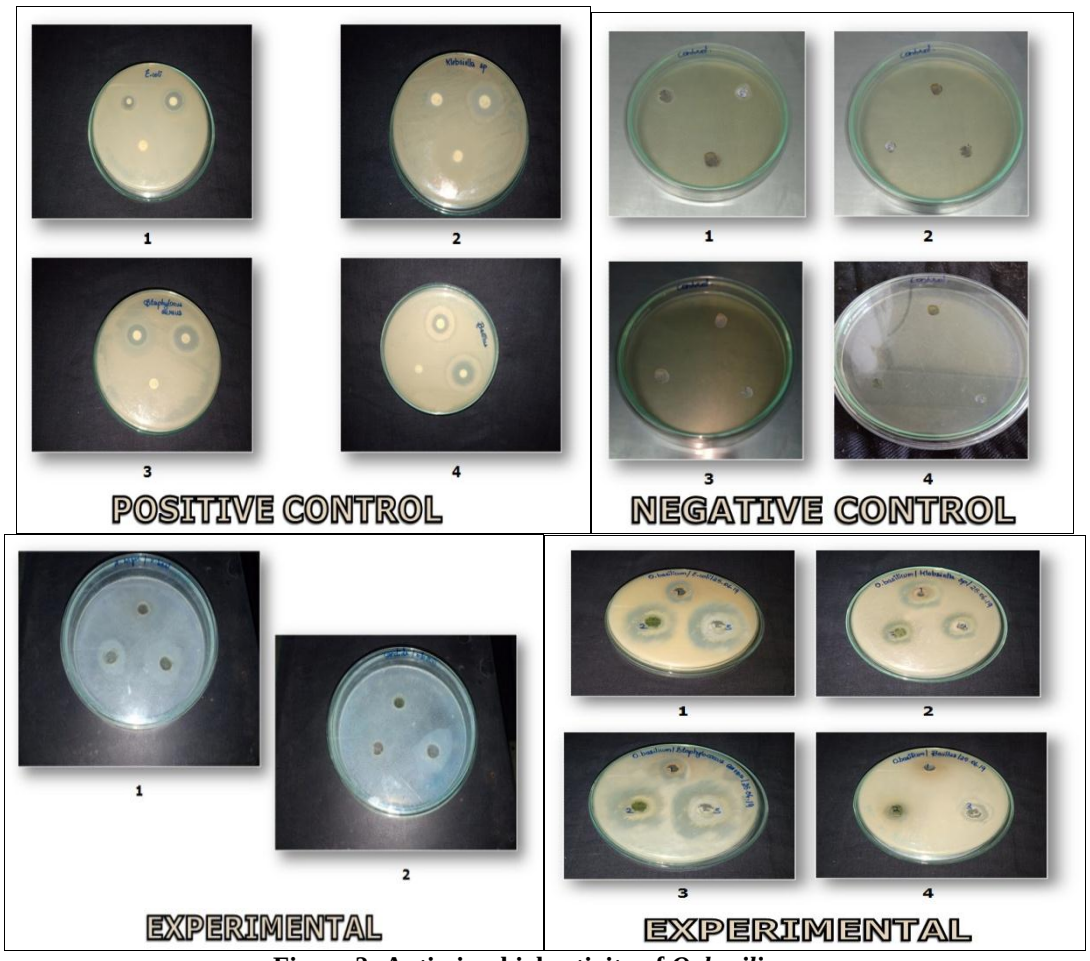

Figure 2: Antimicrobial activity of *O. basilicum*.

Antifungal activity

The plant extracts were tested for their antifungal activity against two fungal strains viz., *Aspergillus niger* and *Candida albicans* on Potato Dextrose Agar plates. The plates were incubated at 37ºC for 48 hours. The Aqueous Extracts show high levels of zone of inhibition when compared to other solvent extracts.

Table 3: Antifungal activity of *O. basilicum*.

	Inhibition Zone diameter in mm (mean ±SD)					
Test Fungi	Hexane		Methanol		Aqueous	
	Experimental	Negative Control	Experimental	Negative Control	Experimental	Negative Control
A. niger	0	–	15	–	17	–
C. albicans	0	–	0	–	10	–

Antioxidant Activity

The free radical scavenging potential of the chosen extracts of *O. basilicum* were tested at varying concentrations and their activity was compared with the reference standard Ascorbic acid. It was observed that maximum radical scavenging activity was exhibited by 500 µl concentration of the aqueous extract with 81% of antioxidant activity while that of the ascorbic acid was found to be 97%.

Table 4: Anti-Oxidant Activity – DPPH of *O. basilicum*.

Anti Oxidant Activity – DPPH						
O. basilicum	Percent Inhibition					
	Sample	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml
	Methanol	31.70%	39.90%	49.10%	64.70%	79.10%
	Aqueous	33.30%	46.70%	47.70%	75.60%	81%
	Hexane	21.10%	27.60%	53.40%	70.94%	73.50%
	Ascorbic Acid	94.10%	95%	96%	96%	97%

CONCLUSIONS

This study has shown that *O. basilicum* contains primary and secondary metabolites that can be pharmacologically useful as well as posses some antibacterial antifungal and antioxidant properties.

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