Pak. J. Biochem. Mol. Biol. 2016; 49(2):36-39

Phytochemical screening and antibacterial activities of essential oil, ethanolic and methanolic extracts of *Ocimum basillicum* L.

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Abstract: In this study phytochemical screening of aqueous extract of *Ocimum basillicum* L. (sweet basil) was done along with the evaluation of antibacterial activities of crude ethanolic, methanolic extracts and soxhlet extracted essential oil against four bacterial strains i.e. *Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Bacillus thuringiensis.* Phytochemical screening showed the presence of carbohydrates, tanins, coumarins and steroids. *Ocimum basillicum* L. showed the antibacterial activities against all four strains by forming zone of inhibition.

Keywords: Antibacterial activity, phytochemical, sweet basil
Received: May 27, 2016
Revised: June 07, 2016 **Accepted:** June 15, 2016
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INTRODUCTION

Ocimum basilicum L. (sweet basil) is called "king of herbs" and belongs to family Lamiaceace[1]. Basil contains many phytochemical compounds which manifest its antioxidant properties and various health benefits. In sweet basil antimicrobial agents are present naturally due to which they show antimicrobial effects against various pathogenic microorganisms[2,3].

The genus of sweet basil, *Ocimum* has more than 65 species but only specie is the basil which is majorly cultivated at commercial level as an essential oil crop in many countries of the world[4]. It is a condimental plant and used as flavoring agent in different dishes worldwide and not only this it has well known medicinal properties[5,6].

Many compounds are present in the essential oil of basil but the main components present in abundance are estragol, linalool and eugenol[7]. The data in the literature shows that linalool is the main active agent which is maximally responsible for its antibacterial activities[8].

Basil is the medicinal plant and had natural bioactive compounds in abundance due to which it had proved very helpful in promoting health activities such as it is antioxidant and also shows antihypertensive and anti-inflammatory activities along with antibacterial activities[9]. Therefore it can be used for the treatment of various diseases such as cardiovascular diseases and to remove the stress[10].

MATERIALS AND METHODS

Plant material

Sweet basil leaves were collected from home lawn and were identified and confirmed by Dr. Najam-us-Saher in Undergraduate department of the University of the Punjab. In this study, basil leaves were collected for the determination of antibacterial activities against gram positive and gram negative bacteria through agar well and disc diffusion method and for the phytochemical screening as well.

Extract preparation

Leaves were washed properly with tap water to remove all the dust and particles and then rinsed with distilled water. After washing, leaves were dried and then grounded in grinder to get fine powder. Twenty five gram of leaves powder was soaked in 100 ml of methanol and 95% ethanol for 5 days at room temperature and for regular infusion, mixture was mixed daily. After 5 days, extract was filtered by using Whatman filter paper No. 1 and then filtrate was dried and concentrated using rotary evaporator. The dried extract was stored in sterile bottle at -20^{o} C for further use.

Oil Extraction

The essential oil of basil leaves powder was extracted by soxhlet extraction method. 25 g of leaf powder was extracted with 250 ml of 95% ethanol and after extraction oil was concentrated by using rotary evaporator after filtering with Whatman filter paper No. 1 and was stored in sterile bottle at -20^{o} C for further use.

Phytochemical screening

Phytochemical testing was done by preparing the aqueous extract of basil leaf powder. Extract was prepared by overnight soaking and constant stirring of 1 g leaf powder in 100 ml of sterilized distilled water at room temperature. Tests were carried out for carbohydrates, proteins, saponins, tanins, steroids,

coumarins, leucoanthocianins and flavonoids as shown in table 1.

Antibacterial activities by disk diffusion and agar well method

Test Organisms

Four strains of bacteria were selected for determining the antibacterial activities. Selected strains were *Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Bacillus thuringiensis.* Those active bacterial strains cultures were taken from the Institute of Biochem. and Biotech., University of the Punjab, Lahore.

Disc diffusion method

For evaluating antibacterial activities of Basil through disc diffusion method, firstly discs of 6mm diameter were prepared by using Whatman filter paper No.1 and were sterilized by autoclaving. Then Luria Broth Agar media was prepared and autoclaved, after autoclaving it was poured in sterilized petriplates and allowed to solidify. 100µl of bacterial culture was spread on solidified nutrient agar plate by using the sterilized spreader. Then sterilized forcep was taken to hold and dipped the disc for 10 seconds in the already prepared extract and then put it in the Centre of the nutrient agar plate. Same procedure was repeated for the positive and negative control plates and plates were incubated for 24 hours at 37^{o}C in the incubator. The zones of inhibition were determined in mm for both ethanolic and methanolic extract and essential oil. To avoid the contamination, whole procedure was carried out in laminar flow hood. Antibacterial potential of basil crude extracts and essential oil against gram positive and gram negative bacteria along with positive control (ampicillin) and negative control (methanol and ethanol only) by disc diffusion method are given in the table 2.

Agar Well Diffusion method

To evaluate the antibacterial activities through agar well diffusion method, first of all Luria Broth Agar media was prepared and sterilized by autoclaving. Then this sterilized media was poured in sterilized petriplates and was allowed to solidify. After solidification, 100µl of bacterial culture was spread with sterilized spreader in the nutrient agar plate and then sterilized cork borer was taken and well was made in the Centre of plate. It was filled with 40µl of extract sample and placed in incubator for 24 hours at 37^{o}C. Same steps were followed for the positive and negative control plates. After 24 hours incubation, zone of inhibition was measured in mm with the help of scale. To avoid the contamination, laminar flow hood was used. Antibacterial potential of basil crude extracts and essential oil against gram positive and gram negative bacteria along with positive control (ampicillin) and negative control (methanol and ethanol only) by agar well method are presented in the table 3.

RESULTS AND DISCUSSION

Phytochemical screening

Results of phytochemical screening of the aqueous extract of *Ocimum basillicum L*. showed the presence of carbohydrates, tanins, coumarins and steroids which are given below in the table 1. These results of phytochemical screening are similar with the results of Adham *et al.,* (2015) in which screening of phytochemical compounds of *Ocimum basillicum L*. extract also showed the presence of carbohydrates, tanins and flavonoids.

Table 1: Phytochemical screening of aqueous extract of Ocimum basillicum L.

Phytochemicals	**Aqueous Extract**
Carbohydrates	+
Proteins	-
Saponins	-
Tanins	+
Coumarins	+
Steroids	-
Flavonoids	+
Leucoanthocianins	-

(+) = Presence (-) = Absence

Antibacterial activities

Results of this study are summarized in table 2 and table 3 which showed that both the essential oil and ethanolic and methanolic crude extract of *Ocimum basillicum* L. has inhibitory effects on the growth of all four strains of bacteria by both agar well and disc diffusion method.

In the disc diffusion assay, essential oil showed maximum antibacterial activities against gram negative bacteria *E.coli* by forming the zone of inhibition of 8 mm, while in case of gram positive bacteria (*S.aureus, B.subtilis* and *B.thuringiensis*) essential oil had given the same zone of inhibition of 4mm diameter. Crude methanolic extract had shown 5 mm zone of inhibition against gram positive bacteria and against gram negative bacteria it had given an inhibition zone of 4mm diameter. Crude ethanolic extract had shown the zone of inhibition of 6mm in diameter against gram negative bacteria while against gram positive bacterial strains it had given inhibition zone of 4mm, 5mm and 6mm against *B.thuringiensis, S.aureus* and *B.subtilis* respectively.

Table 2: Zone of inhibition of essential oil and ethanolic and methanolic extract of Ocimum basillicum L. against bacterial strains by disk diffusion method (mm).

Extracts, Oil and controls	***S. aureus***	***E. coli***	***B. subtilis***	***B. thuringi-ensis***
Methanolic	5	4	5	5
Ethanolic	5	6	6	4
Essential oil	4	8	4	4
Methanol (-)	---	---	---	---
Ethanol (-)	3	3	---	---
Ampicillin (+)	6	5	9	5

Table 3: Zone of inhibition of essential oil and ethanolic and methanolic extract of Ocimum basillicum L. against bacterial strains by agar well method (mm).

Extracts, Oil and controls	***S. aureus***	***E. coli***	***B. subtilis***	***B. Thuringi-ensis***
Methanolic	7	4	6	8
Ethanolic	4	7	4	5
Essential oil	7	6	7	6
Methanol (-)	---	---	---	---
Ethanol (-)	---	---	---	---
Ampicillin (+)	6	7	9	4

In agar well method, essential oil had shown the inhibition zone of 7mm diameter against *S.aureus* and *B.subtilis* while it had given the 6mm inhibition zone against *B.thuringiensis* and *E.coli.* Crude methanolic extract had given the inhibition zone of 4mm, 6mm, 7mm and 8mm against *E. coli, B. subtilis, S. aureus* and *B.thuringiensis.* Crude ethanolic extract had given the same inhibition zone diameter of 4mm against *S. aureus* and *B. subtilis* and it had gviven the inhibition zone diameter of 5mm and 7mm against *B.thuringiensis and E. coli* respectively.

These results showed that essential oil and crude ethanolic and methanolic extracts of *Ocimum basillicum* L. had given almost same zone of inhibition against all four bacterial strains and there is no significant difference in the diameter of inhibition zone in both agar well and disc diffusion method.

Our results showed the similarity with the results of Moghaddam, *et al.*, (2011) in which essential oil extract of *Ocimum basillicum L*. leaves was used to evaluate the antimicrobial activity against pathogenic bacteria i.e. *Staphylococcus aureus, Escherichia coli, Bacillus cereus* and *Pseudomonas euriginosa.* Essential oil showed effective antibacterial activities against these pathogenic bacterial strains. Adam, *et al.,* (2015) also reported the antibacterial activities of ethanolic extract of *Ocimum basillicum* L. leaves against 100 bacterial strains by disc diffusion method. A lot of literature is available in which antimicrobial properties of *Ocimum basillicum L*. are evaluated by using different methods and organic solvents which approved its antimicrobial nature. And now, due to its approved antimicrobial activity and lower risk to the user, *Ocimum basillicum L*. is preferably used in pharmaceutical field for the preparation of the oral, dental and other antimicrobial products.

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