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Research Article

Biochemical potential and screening of bioactive components of *Ocimum basilicum*

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Abstract

Secondary metabolites and phytochemicals are abundantly found in plants with different remedial properties and pharmaceutical benefits with less or no harmful effects. In the history of mankind, *Ocimum basilicum* (sweet basil) is reported to be used as antimicrobial, cardio tonic, and as a remedial agent to treat various symptoms of diabetes. As well as it is used as an anticancer agent. Sweet basil has medicinal potential of treating gastric disorders, relaxing blood vessels, healing wounds and different infections in humans. Current study was designed to analyze the biological capacity of essential oil and methanolic extract of *Ocimum basilicum* leaves to assess the presence of some vital phytochemicals along with its therapeutic and biological potentials. Antioxidant, antifungal, antimicrobial and ant biofilm potential of basil was determined. Fourier transformation infrared spectroscopy **(**FT-IR) and High Performance Liquid Chromatography (HPLC) techniques were followed to analyze some basic functional groups of crude basil powder and to screen some phenolic compounds. This study can be further helpful for pharmaceutical purpose to treat infectious and non-infectious diseases through the natural drug discovery.

Keywords: Antimicrobial; Flavonoids; *Ocimum basilicum;* Phenolics; Phytochemicals; Sweet basil

Introduction

Plants provide us with many important phytochemicals along with secondary metabolites which serve various benefits for health and different medical properties without or negligible side effects. Since ancient times, different herbs have been used as a remedy to treat most of the human illnesses. Economically it is convenient to obtain the natural extracts or products from plants to treat health disorders and certain infectious illnesses **[1]**. Due to medicinal capacity of *Lamiaceae* family plants, they are widely used by mankind. Secondary metabolites of interested research plant (*Ocimum basilicum*) possess the potential of reducing the risks of certain microbial infections due to the presence of active antimicrobial compounds. Essential oils, alkaloids, tannins, steroids, flavonoids, phenols and resins might be included in these secondary metabolites **[2]**. Different basil extracts might be consisted of essential oils, vitamins and poly phenolic

compounds and many other biologically active compounds which exhibit insecticidal, antipathogenic and stress-relieving properties and treats ailments related to respiratory tract, excretory organs, gastric illnesses, skin and eye problems [3].

Medicinal plant, specifically species of *Ocimum* plants are highly ranked due to possession of exhibiting varying properties of curing different diseases [4]. Considerable amount of naturally occuring antioxidants which is contained by *Ocimum basilicum* have protecting effects against numerous degenerative ailments [5].

In drug metabolism, *Ocimum basilicum* is found to have chemomodulatory effects. Therefore, using natural products of plants is highly beneficial against oxidative damage caused by different degenerative and toxic agents and in preventing from side effects of various chemical products [6].

So, therefore the current study was planned with the biochemical prospective of the *Ocimum basilicum* (sweet basil) to explore the medicinal prospectives of essential oil & leaves extract of sweet basil. As well as interpretation of chemical analysis of few essential and biologically active phytochemicals that are capable of rendering *Ocimum basilicum* with many biological activities.

Materials and Methods

Collection of plant sample & pre-treatment of samples

Plant used in this study is *Ocimum basilicum* (sweet basil) which was obtained from the Botanical Garden of University of Agriculture Faisalabad. Only leaves of *O. basilicum* plant were used to investigate their biological potential. Selected plant sample was washed and sterilized. Dried leaves of the plant were then grounded and transferred in air tight glass jar for research.

Extraction of plant samples

Methanol extraction and fractions preparation

Crude extract of grounded leaves was obtained by dissolving in 80% methanol. In order to prevent the loss of essential components and for analytical purpose, basil sample was stored at 4^{o}C [7]. After formulating the methanol extract, it was prepared in three different fractions on the basis of sample concentration as (5%, 10% and 15%) and these concentrations were prepared in dimethyl sulfoxide (DMSO) solution [8].

Essential oil extraction

The aerial parts of the basil plant were collected and submitted to water distillation. Obtained essential oil was dried over Sodium sulfate anhydrous (Na2SO4) and stored at room temperature after filtration, until tested and analyzed [9].

Antioxidant activity

In vitro antioxidant capacity of the selected plant was tested by adopting different assays. The method described by Ainsworth and Gillespie in 2007 [10] was used for the estimation of total phenolic contents (TPC) by using Folin-Ciocalteu reagent, Procedure adopted by Chang and his colleagues in 2006 [11] was followed for the estimation of total Flavonoid Content (TFC) of the leaf extracts of *Ocimum basilicum*, and 2, 2-diphenyl-1-1-picrylhydrazylstable radical (DPPH) assay was performed as described by Noor and his co-workers [12].

Antimicrobial assay of plant extracts

Four bacterial strains were selected to check the antimicrobial effect of sweet basil. These selected bacterial strains were as: *Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Pasturella multocida* and two fungal strains i.e. *Rhizopus solani* and *Aspergillus Niger*. Antimicrobial activity of the selected plant samples was tested by following Kirby-Bauer disc diffusion method. All of the microbial strains were locally isolated and were procured from Medicinal Biochemical Laboratory, Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan.

Determination of antibacterial and antifungal activity

The discs of plant extracts and standard

solution as well, both were applied on sterilized nutrient agar and pressed lightly [13]. After incubation, the petri dishes were taken out and then areas of growth inhibition (in millimeter) were measured with the help of ruler to assess the antifungal activity by disc diffusion assay [14]. Petri dishes were prepared in triplicates.

Minimum inhibitory concentrations (MIC) & antibiofilm assay of plant extracts

Resazurin microtitre-plate assay was used to evaluate the Lowest Inhibitory Concentration of basil extracts [15] and the quantitative assay for determining the antibiofilm capacity of *O. basilicum* extracts was checked by following the method which described by Dheepa and his colleagues [16].

High performance liquid chromatography (HPLC) of plant extract

Phenolic acids were identified and quantified through High Performance Liquid Chromatography (HPLC). 500 mg dried leaf powder was ground in particle size of 0.125–0.250 mm. immersed in 5 ml of methanol of HPLC grade and left at 4 °C overnight in darkness. Decantation and filtration of supernatants was done through syringe filter [17] and then samples were shifted to HPLC vials.

Fourier transformation infrared spectroscopy (FT-IR)

FT-IR assay was executed on crude basil powder sample to identify its functional groups. Infrared spectral studies were carried out on FT-IR spectrometer in the presence of dry air, and it was performed at room temperature. In Potassium bromide (KBr) on the FT-IR spectrophotometer using KBr pellet, the spectra from the samples were measured [18].

Statistical analysis

Values of each measurement are expressed as mean ± Standard deviation (SD). Data was checked by using one way ANOVA with the use of Minitab 17 software [19].

Results and Discussion

Current study was planned to investigate the biochemical potential of sweet basil (*Ocimum basilicum*) along with the screening of some important phytochemicals through biological assays and other analytical techniques. Many biological properties of *Ocimum basilicum* extracts were identified as; antioxidant activity and antimicrobial potential as well as antibiofilm potential. The most imperative part of our research work was the separation of phenolic acids through HPLC and analyzing crude basil powder by using the FTIR technique.

2, 2-diphenyl-1-1-picrylhydrazylstable radical (DPPH) scavenging assay

DPPH is a constant free radical usually used to find out the capability of compounds to scavenge free radicals. So, in this study, DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical was used to assess the *In-vitro* antioxidant capacity of methanolic extract of *Ocimum basilicum* and among varying concentrations of essential oil. The results of antioxidant activity of *Ocimum basilicum* methanolic extract and essential oil extract are shown in (Fig. 1) which depicted that at 100% concentration the essential oil has 93.5% maximum antioxidant activity as compared to methanolic extracts and their radical sifting activity is directly proportional to their concentration. As Shafique and his colleagues in 2011 [20] reported that basil essential oil extracts have higher antioxidant activity at 100% concentration and showed 96.16% activity by DPPH scavenging effect. So, they concluded that the essential oil extract of basil was more effective than Butylated hydroxytoluene (BHT).

Total phenolic content (TPC)

Linear regression gallic acid curve, a standard phenol ($y = 0.0055x + 0.0987$) was used to determine the Phenolic Content of the methanolic extract of sweet basil leaves and results were shown in mgGAE/g of dried extracted samples weight. TPC results in (Fig. 2) clearly show that at 15% concentration phenolic content of the

samples are 20 GAEmg/g that is directly related to the basil extract concentration. Results of various methanolic extract fractions of *O. basilicum* were compared to the published results of Uyoh and his fellows [21] in which they recorded 27.41 µgGAE/mg of mean TPC of leaves methanolic extract.

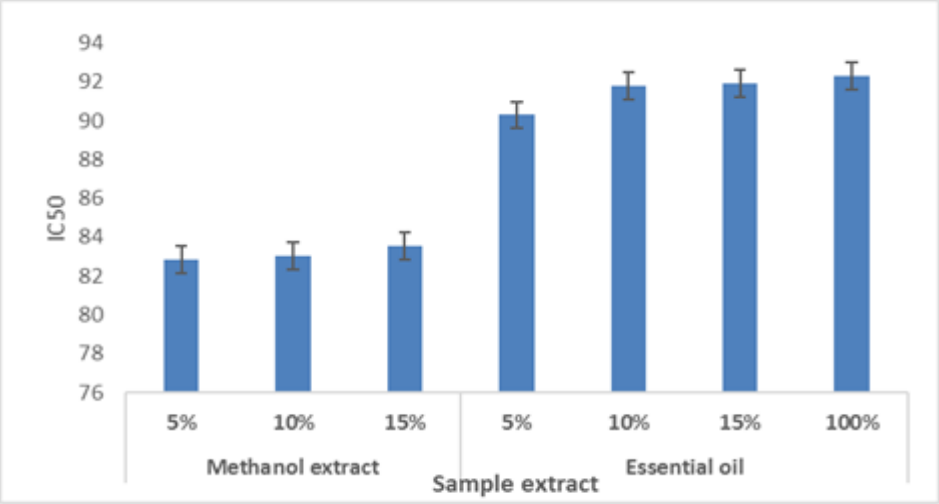

Figure 1. Percentage of free DPPH radical sifting activity of the sweet basil methanolic and essential oil extracts

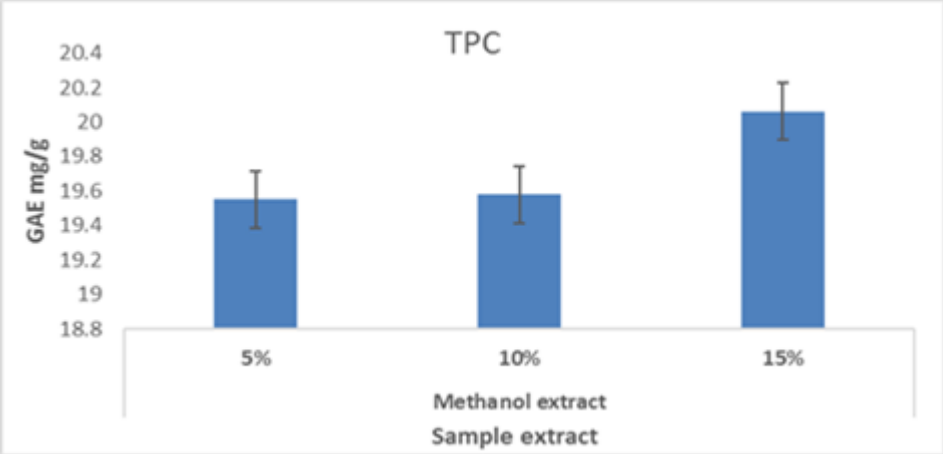

Figure 2. TPC of basil Methanolic Leave extracts. Each methanolic extract concentration shows the GAEmg/g value

Total flavonoid content (TFC)

Results of (Fig. 3) clearly show that total flavonoid content (TFC) of methanolic extracts of *Ocimum basilicum* leaves fall in the same range i.e., resulted values are close enough to one another and flavonoid content of samples rises with the increasing concentration of samples. Reported results of Vidovic and his coworkers [22] were taken as a reference to compare the obtained values of current study. They reported that *Ocimum basilicum* with total flavonoid content in the range of 4.28gCE/100g to 5.10gCE/100g while 70% ethanol was used as a solvent.

Antimicrobial activity

Disc diffusion method revealed that essential oil extracts of basil with varying concentrations have antibacterial activity contrary to all the taken strains of gram negative and gram positive bacteria. Although 5% fraction of basil essential oil and concentrated basil oil extracts have inhibition zones that are ranging from

14mm to 45mm against *S. aureus* and *B. subtilis* respectively while methanolic extracts of basil did not show any antimicrobial activity. Current antimicrobial study was interrelated with the research work of Adiguzel & his colleagues [23] and almost same results were found in both studies. 100% concentration of basil essential oil exhibited the maximum antibacterial potential in contradiction of all the selected four bacterial strains (*Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Pasturella multocida)* whereas three lower concentrations of basil oil extracts were shown with the minimum inhibition zones of bacterial growth. After confirmation of antibacterial activity, different fractions of sweet basil essential oil were checked for their minimum inhibitory concentration (MIC) against the bacterial strains, already mentioned in the current study. MIC (Fig. 4 & Table 1) shows comparable difference among the minimum inhibitory concentration of the control i.e., Rifampicin and the particular basil essential oil fractions. 100% concentration of basil essential oil fraction was seen with the MIC in the range of 2.46µg/mL to 76.34µg/mL for *B. subtilis* and *E.coli with* 5% fraction of basil essential oil respectively. Minimum Inhibitory Concentration (MIC) results of present research work were matched to that of the reported outcome of Hamdan and his co-workers [24]. Both studies were presented with the same results in which the values were lying close to each other and almost within the same ranges.

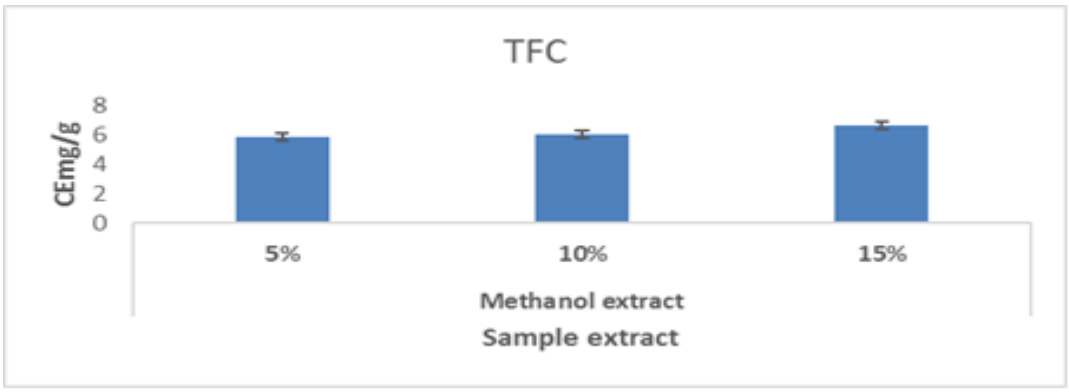

Figure 3. TFC of basil methanolic leave extracts. Each methanolic extract concentration shows the CEmg/g value

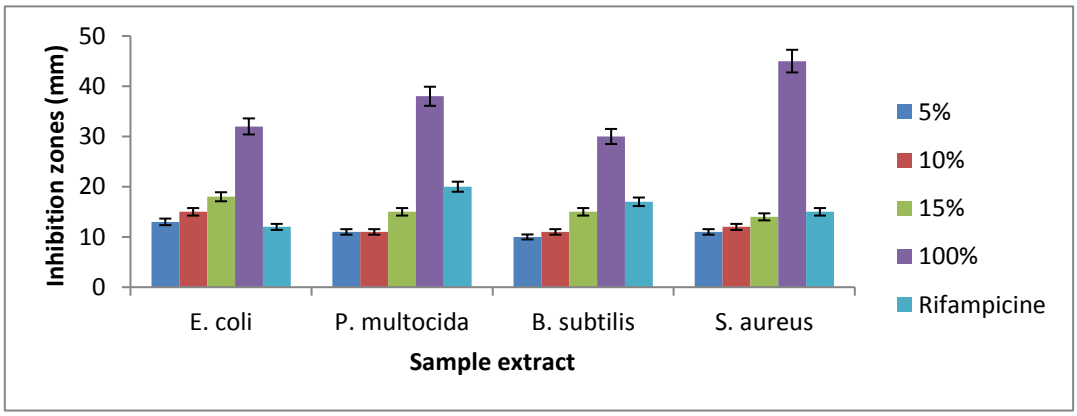

Figure 4. Basil essential oil showed antibacterial activity against selected bacterial species

Table 1. Result shows the minimum inhibitory concentration of *Ocimum. basilicum* essential oil with the use of Rifampicin as a positive control

Plant specie extract	Sample fraction	Minimum Inhibitory Concentration(µg/mL)			
		Staph aureus	***Bacillus subtilis***	***Escherichia coli***	***Pasteurella multocida***
***Ocimum basilicum* essential oil**	5%	33	28.94	76.31	55.21
	10%	31.33	13.05	58.86	47.82
	15%	27.58	3.20	45.73	10.57
	100%	14	2.46	45.73	6.23
Rifampicin	100%	12	2.35	19.52	4.90

Similarly, all the three fractions of methanolic extract of sweet basil and 100% concentration of essential oil extracts were checked for antifungal activity against two fungal strains as: *Aspergillus Niger* and *Rhizopus solani.* Basil essential oil fractions was observed with the significant antifungal activity against the both *Aspergillus niger* and *Rhizoctonia solani.* Whereas fractions of basil methanolic extract did not display antifungal activity as given in (Table 2).

***Ocimum basilicum* Antibiofilm activity**

Methanolic extract of *O. basilicum* and all of its essential oil divisions were evaluated for their antibiofilm potential through micotitre plate assay describe by Dheepa and his fellows. DMSO (dimethyl sulfoxide) was taken as a negative control. *Staphylococcus aureus* and *Escherichia coli* were selected for inhibition of biofilm formation against them. All of the basil essential oil fractions were shown with appreciable intensity of inhibiting biofilm production with the maximum inhibiting activity seen in the concentrated basil essential oil fraction with the supreme value of 55% against *S. aureus* and 40% against *E. coli.* Whereas methanolic fractions of sweet basil did not show any antibiofilm potential as shown in (Table 3). In 2014 Fernenda [25] and his colleagues checked the inhibition of *S. mutants* biofilm formation by basil extract and appreciable inhibition was done by basil extract. Different studies were carried out on different bacterial strains but less reported data was available on *E. coli* and *S. aureus* strains that is used in current study. Hence this study showed the significant antibiofilm activity by basil oil against *S. aureus* and *E. coli which* provides the basis for further research purpose.

Documentation of antioxidant compounds through HPLC in crude methanolic extract of *Ocimum basilicum*.

High Performance Liquid Chromatography (HPLC) was performed to quantify the amount of total phenolic compounds in the crude methanolic extract of *Ocimum basilicum.* (Table 4) quantifies different phytochemicals and their (%) Quantities like caffeic acid (0.004868), Gallic acid (0.0015), Cinamic acid (0.000896), Quercitin (0.000036) and chromatotropic acid (0.000411). In 2008 Kruma and his colleagues have been observed that basil contain different phenolics and the total phenolic components. They were found to be 12.18mg/100g of phenolic compound through crude methanolic extract [26]. Rosmarinic acid, a derivative of phenolic acid was the only one phenolic compound that was analyzed in that study. Flavonoid was not identified through HPLC whereas present study showed that basil have 0.36ppm of quercitin as we can observed in the HPLC chromatogram of (Fig. 5).

Interpretation of Fourier Transform Infrared Spectroscopy (FTIR) spectral data

Through FTIR wave number, % transmittance**,** peak values and corresponding presumptive functional groups of the coarse powder of *O.basilicum* were determined that are presented in (Fig.

6 & Table 5). Characteristic absorption bands of crude sweet basil powder are shown in FTIR spectrum within the range of 1350cm^{-1} to 3400cm^{-1} that depict the presence of alkanes and amines or amides. Various functional groups were found between these two peak values which were found in the frequency ranges of 1600cm^{-1} for C-C stretch, 2100cm^{-1}for C≡C stretch and 2850cm^{-1} for C-H stretch at their peculiar peaks.

Table 2. Antifungal activity of the *ocimum basilicum* against *A. Niger and R. Solani*

Plant Specie extract	Sample fraction	Zones of growth inhibition in mm	
		A. Niger	***R. solani***
***Ocimum basilicum* methanolic extract**	5%	-	-
	10%	-	-
	15%	-	-
***Ocimum basilicum* essential oil**	100%	8^{a}	15^{b}
Fluconazol	Methanol	6^{b}	18^{a}

Table 3. Antibiofilm activity of *Ocimum basilicum with Methanolic and essential oil fractions*

Plant Specie extract	Sample fraction	Biofilm inhibition (%)	
		S. aureus	***E. coli***
***Ocimum basilicum* ethanolic extract**	5%	-	-
	10%	-	-
	15%	-	-
***Ocimum basilicum* essential oil**	5%	20^{d}	14^{d}
	10%	29^{c}	22^{c}
	15%	39^{b}	31^{b}
	Conc.	55^{a}	40^{a}
DMSO	100%	-	-

Table 4. Identification of phenolic compounds in sweet basil

Plant specie	Phytochemical	Quantity (%)
Basil crude extract	Caffeic acid	0.004868
	Gallic acid	0.0015
	Cinamic acid	0.000896
	Quercitin	0.000036
	Chromatotropic acid	0.000411

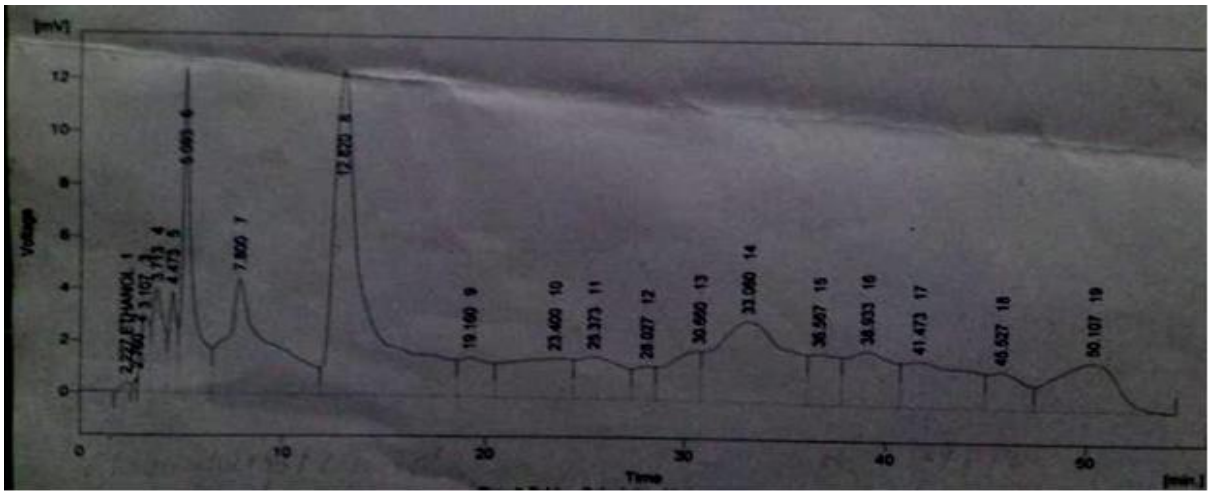

Figure 5. HPLC chromatogram of crude methanolic extract of sweet basil

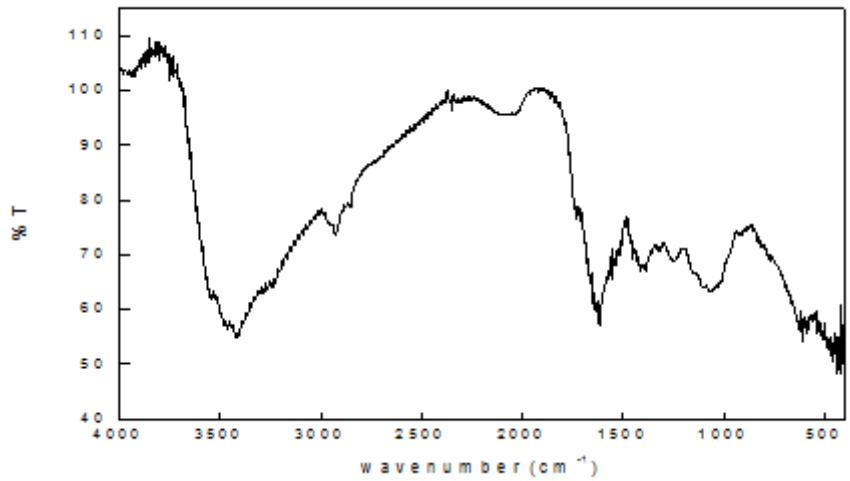

Figure 6. FTIR spectrum of Ocimum *basilicum crude powder* shows the wave number and % transmittance

Table 5. Peak values of IR spectrum and functional groups of basil crude powder

Plant sample	Frequency cm^{-1}	Bond	Functional group
Basil crude powder	3250-3400	N-H stretch	1^o or 2^o amines or amides
	2850-3000	C-H sretch	Alkanes
	2100-2260	C≡C stretch	Alkynes
	1585-1600	C-C stretch	Aromatics
	1350-1370	C-H rock	Alkanes

Conclusion

This study decisively estabilished the worth of basil (*Ocimum basilicum*) essential oil extracts against gram negative and gram positive bacterial strains, showing that this plant with some important phytochemicals, functional groups, primary & secondary amines and certain organic compounds that have potential against bacterial defense. Hence the results of present study justify that further investigations should be carried out on *Ocimum basilicum* for the identification and purification of bioactive compounds. Positive results from subsequent studies would certainly strengthen its potential as a cost-effective and novel agent against multi drug resistant bacteria.

Authors' contributions

Conceived and designed the experiments: M Shahid, Performed the experiments: U Nazir, Analyzed the data: U Nazir & S Javaid, Contributed materials/ analysis/ tools: U Nazir & M Shaid, Wrote the paper: U Nazir S Javaid H Awais & F Bashir.

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