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### RESEARCH ARTICLE

#### PHYTOCHEMICAL, TOTAL PHENOLIC CONTENTS, AND ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *OCIMUMBASILICUM* L. LEAF EXTRACT IN AL-BAHA AREA, SAUDI ARABIA

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#### Abstract

The aim of this study is to determine the phytochemical and total phenolic contents in addition to investigating the antioxidant and antimicrobial activities of *Ocimumbasilicum* leaf extracts. The leaves were air-dried and extracted with ethanol, petroleum ether, chloroform and ethyl acetate. The contents of phytochemicals and total phenols were determined in addition to determining the antioxidant and antimicrobial activities. The results showed that ethanol, petroleum ether, chloroform and ethyl acetate extracts contained flavonoids, terpenoids, steroids, tannins, saponins and reducing sugars. The petroleum ether extract had the highest total phenolic content (182.90%), followed by chloroform (171.67%), ethanol (166.03%) and ethyl acetate (106.33%) extracts. DPPH scavenging activity was highest in ethyl acetate extract (46.00%) and lowest in ethanol extract (29.93%). The bacteria *Escherichia coli* and *Staphylococcus aureus* were resistant to ethanol, petroleum ether, chloroform and ethyl acetate extracts, while *Pseudomonas aeruginosa* was resistant to all extracts except chloroform, and *Bacillus cereus* was resistant to ethanol and ethyl acetate extracts. *Candida albicans* was resistant to all extracts except ethanol. The study concluded that, although *O. basilicum* was not active against most microorganisms tested, it had appreciable concentrations of phytochemicals, total phenols, in addition to antioxidant activity.

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#### Introduction:-

Herbs are considered as an ancient source of medicine, flavouring, beverages, dyeing, fragrances and cosmetics which attracted biotechnology, cosmetics, pharmaceutical and food industries (Zaidi and Dahiya, 2015). Different parts of medicinal plants were used since prehistoric times for healing specific diseases due to presence of some bioactive compounds such as alkaloids, flavonoids, essential oil, glycosides, tannins, terpenoids, steroids and others (Bharathi et al., 2014). There is an increasing interest in natural substances which exhibit antimicrobial and antioxidant properties from essential oils and plant extracts supplied to human and animal organisms as food components or specific pharmaceuticals (Shafique et al., 2011). The beneficial medicinal effects of plant materials result from the secondary metabolites such as alkaloids, steroids, tannins, phenol compounds, flavonoids, steroids,

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resins, fatty acids and gums which are capable of producing definite physiological action in the body (Mishra and Mishra, 2011).

In the last decades, although pharmacological industries have produced a number of new antibiotics, the microbial resistance to these antibiotics has increased due to genetic ability of the bacteria to acquire and transmit the resistance against therapeutic agents (Del Campo et al., 2000). Synthetic additives have been widely used, and the trend is to decrease their use because of the growing concern among consumers about such chemical additives, consequently, search for natural additives, especially of plant origin, has notably increased in recent years (Rozmani and Jersek, 2009). Therefore, the development and application of natural products with both antioxidant and antibacterial activities may be necessary and useful to prolong their storage shelf life and prevent food diseases (Rozmani and Jersek, 2009).

*O. basilicum* is widely distributed in tropical and warm temperate regions of the world, being a plant with extraordinary medicinal properties containing several antioxidant compounds (Adam and Omer, 2015). In traditional medicine, *O. basilicum* has been used as an antiseptic, preservative, sedative, digestive regulator and diuretic agent, therefore, is recommended for the treatment of headache, cough, infections of upper respiratory tract, kidney malfunction and to eliminate toxins (Ahmad et al., 2015). The plant is effective in the treatment of stomach problems, fever, cough, gout; and it is administered internally to treat cystitis, nephritis and in internal piles; and the infusion of basil seed is used to treat gonorrhoea, chronic diarrhoea and dysentery (Bilal et al., 2012).

The Arabian Peninsula is the birth place of herbal drugs, where the use of folk medicine has existed since ancient time. However, traditional medicine occupies a significant part of Saudi Arabia's heritage and it is widely practiced until now (Al-Essa et al., 1998). The Kingdom of Saudi Arabia is gifted with a wide range of flora consisting of a large number of medicinal herbs, shrubs and trees (El-Shabasy, 2016). This study was conducted to investigate the phytochemical and total phenolic contents, in addition to antioxidant antimicrobial activities of *O. basilicum* leaf extract collected from A-Baha area, Saudi Arabia.

## Materials and Methods:-

### Sample Collection and Preparation:

Fresh leaves of *O. basilicum* L. were collected from Al-Baha area, Kingdom of Saudi Arabia during the period April-October, 2019. The plant was taxonomically identified and authenticated by Dr. Haidar Abd Algadir, Department of Biology, Faculty of Science, Al-Baha University, where the voucher specimen was deposited for future reference. The leaves were washed with fresh water to remove the soil and dust particles, and subjected to air-drying under shade for three weeks until they were completely dried, then ground into fine particles using an electric grinder.

### Preparation of Ethanolic Extract of Samples:

Two hundred grams (200 gm) of powdered sample were weighed into a clean flask (3 L size). Two thousand milliliters (2000 ml) of 80% ethanol (800 ml ethanol+200 ml distilled water) were added. The mixture was soaked for 2 days at room temperature, then filtered with filter paper (Whatman no. 4). This procedure was repeated three times to ensure that all contents were extracted with ethanol. The filtrates were collected and allowed to air dry for 10 days under shade, and the extract was stored in coloured bottles at 4-6°C till analysis.

### Fractionation of the Ethanolic Extract by Liquid-liquid Extraction:

The ethyl alcohol extract was soaked in 200 ml distilled water and extracted consecutively with different solvents (petroleum ether, chloroform and ethyl acetate, respectively) for 7 days at room temperature to obtain extracts. The filtrates were dried by using a rotary evaporator at room temperature, and stored at 4-6°C till used.

### Total Phenolic Content (TPC):

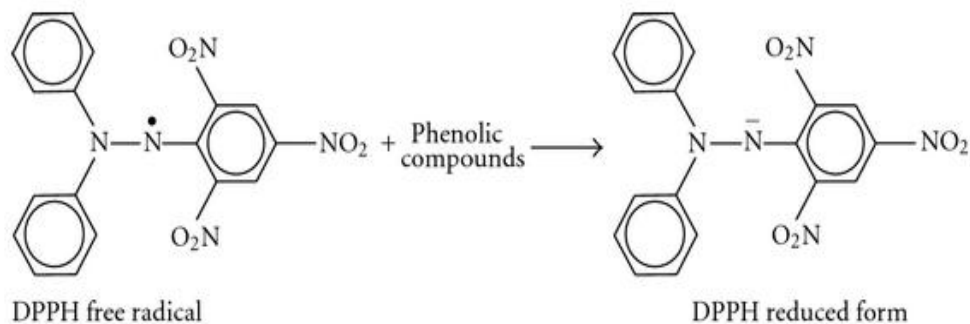
The phenolic content of leaf extracts was determined by a spectrophotometric method (Singleton et al., 1999). Sample solutions of the extracts in the concentration of 1 mg/ml were used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of sample solutions of fractions, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% NaHCO<sub>3</sub>. Blank was consequently prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of NaHCO<sub>3</sub>. The samples were incubated at 30°C for 90 min, followed by determining the absorbance by a spectrophotometer at  $\lambda_{max} = 765$  nm. The samples were prepared in triplicate for each analysis. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolics was read

(mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GAE/gm of extract).

### Total Antioxidant Activity:

#### Free Radical Scavenging Assay:

The antioxidant assay is based on scavenging ability of the antioxidant (s) in plant extracts towards the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), which is deep purple in colour, to form the corresponding hydrazine with accompanying colour change to light purple or golden yellow.



#### Free Radical Scavenging Procedure:

This method was carried out according to Shyur et al. (2005). Stock solution was prepared by dissolving 1mg of the sample in 1ml of absolute ethanol (98%). The stock solution was diluted to final concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.5625  $\mu\text{g/ml}$  in ethanol. Tris-HCl (0.9ml) and 1ml of 0.1 mM DPPH in methanol solution were added to each concentration and incubated at room temperature in the dark for 30 min. The absorbance of the resulting mixture was measured at 517 nm and converted to percentage antioxidant activity using the following formula:-

$$\text{Scavenging activity (DPPH scavenged) (\%)} = \frac{(Ac - As)}{Ac} \times 100$$

Where: Ac= Absorbance of control; As = Absorbance of sample extract

A solution of 0.9 ml Tris-HCl+0.1ml absolute ethanol+1ml absolute ethanol was used as blank, while solution of 0.9 ml tris-HCl+0.1ml absolute ethanol+1ml DPPH was used as control. Freshly prepared DPPH solution exhibits a deep purple colour with a maximum absorbance at 517 nm. The purple colour disappears when an antioxidant is present in the medium, thus, the change in the absorbance of the reduced DPPH is used to evaluate the ability of the compound to act as a free radical scavenger.

#### Antimicrobial Activity Test:

##### Microbial Organisms:

Bacterial strains of *Bacillus subtilis* (NCTC 8236), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), in addition to a fungal strain *Candida albicans* (ATCC 7596) were used for antimicrobial activity test.

##### Preparation of Bacterial Suspensions:

Aliquots (1 ml) of a 24 hr broth culture of the bacteria were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hr, followed by harvesting and washing off the growth with 100 ml sterile normal saline to produce a suspension containing  $10^8$ -  $10^9$ cfu/ml, which was stored at 4°C till used. The average number of viable organisms/ml of the stock suspension was determined using the surface viable counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were made in a sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by a micropipette onto the surface of dried nutrient agar plates, which were allowed to stand for 2 hr at room temperature for the drops to dry and then incubated at 37°C for 24 hr. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and the dilution factor to get the viable count of the stock suspension, expressed as colony forming units (cfu)/ml suspension. Each time a fresh stock suspension was prepared. All the above

experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

#### **Preparation of Fungal Suspension:**

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with a sterile normal saline and suspended in 100 ml sterile normal saline and stored at 4°C until used.

#### **Agar Disc Diffusion:**

The disc diffusion method was used to test the antimicrobial activity of plant extracts by Mueller Hinton agar (MHA) and Sabouraud dextrose agar (SDA) according to Boudjema et al. (2018). Bacterial and fungal suspensions were diluted with a sterile physiological solution to  $10^8$  cfu/ml (turbidity = McFarland standard 0.5). One hundred microliters (100  $\mu$ l) of the bacterial and fungal suspensions were swabbed uniformly on the surface of MHA and SDA, and the inoculum was allowed to dry for 5 min. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of MHA and SDA and soaked with 20  $\mu$ l of each plant extract solution. The inoculated plates were incubated at 37°C for 24 hr in an inverted position. After incubation, the antimicrobial activity was determined by measuring the diameter of the inhibition zone surrounding each disc, and the results were expressed as the diameter of inhibition zone as follows: <9 mm zone (resistant strain); 9-12 mm (partially sensitive strain); 13-18 mm (sensitive strain); >18 mm (very sensitive strain).

#### **Statistical Analysis:**

The statistical analysis was performed using Statistical Analysis Systems (SAS, Ver. 9, SAS Institute Inc., Cary, NC, USA) and the results were presented as the mean $\pm$ standard deviation (mean $\pm$ SD) of three replicates. All data were statistically assessed using the General Linear Model (GLM) and the significant difference was performed using Duncan multiple range test at  $P\leq 0.05$ .

## **Results and Discussion:-**

#### **Phytochemical analysis:**

Phytochemicals present in many herbs received much attention in recent years due to their health benefits (i.e. antioxidant and anti-inflammatory activities). The results of phytochemical screening test of *O. basilicum* showed the occurrence of flavonoids, tannins, terpenoids, steroids, saponins and reducing sugars in ethanol, chloroform and n-butanol extracts, while terpenoids, steroids, saponins and reducing sugars are present in ethyl acetate extract (Table 1). These results are consistent with those of Sriram et al. (2019) who reported that the qualitative analysis of *O. basilicum* leaf extract showed the presence of flavonoids, alkaloids, glycosides, phyosterol, phenols, tannins, proteins, saponins, sterols and triterpenes. Similar results were reported by Sundaraju et al. (2014) who confirmed the presence of various phytoconstituents like alkaloids, flavonoids, phenols, coumarins, tannins, triterpenoids, phytosterols and saponins in the ethanolic extract of *O. basilicum* leaves. Presence of flavonoids, tannins, terpenoids, steroids, saponins and reducing sugars is in agreement with previous studies (Sanni et al., 2008; Chioma et al., 2014; Adam and Omer, 2015; Złotek et al., 2016). Azam and Irshad (2016) documented the presence of carbohydrates, tannins, coumarins and steroids in the aqueous extract of *O. basilicum* L. leaves. The phytochemical analysis of the crude extract revealed the presence of alkaloids, tannins, flavonoids, cholesterol, terpenoids, glycosides, cardiac glycosides, phenols, carbohydrates and phlobatannins, and the absence of saponins and proteins (Gebrehiwot et al., 2015). Isa et al. (2018) reported that the phytochemical screening test confirmed the presence of flavonoids, cardiac glycosides, tannins, saponins and phenols in both aqueous and methanolic extracts of *O. basilicum* leaves. These phytochemicals reduce oxidation, stimulate the immune system against viruses, bacteria and other disease-causing agents and slow the growth of cancer cells; and in plants, they promote physiological survival of plant by protecting it from fungal infections and UV radiations (Isa et al., 2018).

#### **Total phenolic content:**

The total phenolic content (TPC) of *O. basilicum* leaf extracts was determined using the Folin-Ciocalteu phenol reagent. The results revealed no significant ( $P>0.05$ ) difference between ethanol, petroleum ether, chloroform and ethyl acetate extracts, although the highest content ( $182.90\pm 107.80$  mg GAE/gm) was recorded in petroleum ether extract, while the lowest ( $106.33\pm 57.90$  mg GAE/gm) was recorded in ethyl acetate extract (Table 2). It was announced that the total phenolic content of six different extracts of *O. basilicum* varied from 0.408 mg GAE/gm dry weight to 0.881 mg GAE/gm dry weight (Aburigal et al., 2017). Gradinariu et al. (2013) analyzed the total phenolic content of *O. sanctum* and *O. basilicum* L. and confirmed that *O. sanctum* had the highest polyphenols (28.99 mg

caffeic acid/gm dry extract), while *O. basilicum* L. had the lowest (17.17mg caffeic acid/gm dry extract). The total phenolic content of ether, chloroform, ethyl acetate, n-butanol and aqueous extracts of *O. basilicum* aerial parts was found to be  $4.86 \pm 0.03$ ,  $4.21 \pm 0.01$ ,  $9.76 \pm 0.03$ ,  $8.45 \pm 0.02$  and  $11.88 \pm 0.02$  mg GAE/gm, respectively (Kaurinovic et al. (2011). Akah et al. (2017) analyzed the total phenolic content of water and ethanol extracts of the fresh and dried *O. basilicum* leaves and reported that the content of aqueous extract of dried *O. basilicum* leaves (1.55 mg GAE/100 ml - 2.25 mg GAE/100 ml) was significantly ( $p < 0.05$ ) higher than the fresh *O. basilicum* leaves (1.13 mg GAE/100 ml), but sun-dried, oven-dried at  $50^\circ\text{C}$  and oven-dried at  $80^\circ\text{C}$  leaf extracts were significantly ( $p < 0.05$ ) lower than the oven-dried leaf extract at  $60^\circ\text{C}$ . The content of ethanol extract of shade dried leaves (2.04 mg GAE/100 ml) was significantly ( $p < 0.05$ ) higher than the oven dried leaves (1.25 mg GAE/100 ml - 1.58 mg GAE/100 ml) and fresh leaves (1.0 mg GAE/100 ml), but that of fresh leaves did not differ significantly ( $p > 0.05$ ) from those of sun, solar and  $50^\circ\text{C}$  oven-dried samples. The total phenol contents of the leaf extracts of *O. basilicum* and *O. gratissimum* were estimated quantitatively from a linear regression curve ( $y = 0.4982 + 0.0101x$ ,  $r^2 = 0.948$ ) of Gallic acid, a standard phenol, and expressed in micrograms Gallic acid equivalents per milligram of sample ( $\mu\text{g GAE/mg sample}$ ), and the results showed that *O. basilicum* leaf extract had a mean total phenol content of 27.41  $\mu\text{g GAE/mg}$  which was significantly higher ( $p < 0.01$ ) than that of *O. gratissimum* with a mean value of 9.09  $\mu\text{g GAE/mg}$  (Uyoh et al., 2013). Sarfraz et al. (2011) reported that the maximum total phenolic content of methanol extract of *O. basilicum* seeds was  $5.67 \pm 0.77$  mg GAE/100 gm, while the minimum value ( $3.37 \pm 0.03$  mg GAE/100 gm) was observed for hexane extract of *O. basilicum* leaves. Phenolic compounds constitute one of the most diverse and widespread groups of natural compounds, having a broad spectrum of biological activities including antioxidant and radical scavenging properties, and that the high amounts of phenols and flavonoids in extracts may explain their high antioxidant activities (Uyoh et al., 2013).

#### Antioxidant activity:

DPPH radical scavenging assay provides an easy, rapid, and convenient method to evaluate antioxidants and radical scavengers, which is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolourize in the presence of antioxidants (Uyoh et al., 2013). The antioxidant activity of *O. basilicum* leaf extracts was determined using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) as a free radical scavenger. The results showed that the highest antioxidant activity was in ethyl acetate extract ( $46.00 \pm 6.85\%$ ), followed by chloroform ( $44.10 \pm 10.28\%$ ), petroleum ether ( $32.05 \pm 7.97\%$ ) and ethanol ( $29.93 \pm 9.51\%$ ) extracts, although the difference was not significant (Table 3). The results in this study are lower than those of Sarfraz et al. (2011) who concluded that the maximum DPPH free radical scavenging activity (84.59%) was shown by methanol extract and the minimum activity (57.35%) was shown by hexane extract of *O. basilicum* seeds. Uyoh et al. (2013) reported that *Ocimum* species showed high DPPH radical scavenging activities, with *O. basilicum* scavenging being close with the reference compounds, a finding which validates these plants as powerful radical scavengers that may find application as good natural antioxidants. These findings are attributed to its composition, being a rich source of polyphenols, flavonoids and rosmarinic acid, which have well-known antioxidant activity (Uyoh et al., 2013). Aburigal et al. (2017) notified that DPPH values of different samples varied from 89.22% to 69.33%, with the highest antioxidant activity being in samples from Maldives, while the lowest antioxidant activity was found in samples from Thailand, and the antioxidant effectiveness is probably due to a relatively high content of methyl-eugenol in *O. basilicum*, in addition, the antioxidant activity of ethyl acetate extract was higher than ethanol extract. Guez et al. (2017) confirmed that *O. basilicum* extract acts as an antioxidant and effectively overcomes the effects of high oxidizing agents such as hydrogen peroxide.

#### Antimicrobial activity:

Table 4 shows the antibacterial activity of *O. basilicum* leaves extracted with ethanol, petroleum ether, chloroform and ethyl acetate against all tested microorganisms at the concentration of 100 mg/ml. The results revealed that *E. coli* and *S. aureus* were resistant to ethanol, petroleum ether, chloroform and ethyl acetate extracts, while chloroform extract showed antibacterial activity against *P. aeruginosa* (10 mm inhibition zone) and both chloroform and petroleum ether extracts showed antibacterial activity against *B. cereus* (13 mm and 10 mm inhibition zones, respectively). The results of resistance of *E. coli* and *S. aureus* reported in this study are confirmed by Kaya et al. (2008) who outlined that *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were resistant to chloroform, acetone and methanol (suspended with 10 ml deionized water) leaf extracts of *O. basilicum*, and Adam and Omer (2015) who found the lowest antibacterial activity to be against *S. aureus* (4.4 mm inhibition zone). Similar results were reported by Azam and Irshad (2016) for ethanolic and methanolic crude extracts of *O. basilicum* L. (inhibition zone of 4-6 mm for gram-negative and gram-positive bacteria). The results are in disagreement with Bharathi et al. (2014) who reported that the highest antimicrobial activity of methanol extract of *O. sanctum* at concentrations of 100

mg/ml, 200 mg/ml and 300 mg/ml was observed against *B. subtilis* (24 mm, 28 mm and 34 mm, respectively) followed by *P. aeruginosa* (25 mm, 30 mm and 31 mm, respectively), *S. aureus* (21 mm, 23 mm and 25 mm, respectively) and *S. pyogenes* (8 mm, 13 mm and 15 mm, respectively). Adam and Omer (2015) found the highest antibacterial activity of *O. basilicum* leaf extract to be against *E. coli* and *P. aeruginosa* (7.8 mm inhibition zone each). Adigozel et al. (2005) reported that the ethanol extract of *O. basilicum* has an antimicrobial effect against *Acinetobacter*, *Bacillus*, *Escherichia*, and *Staphylococcus*, while the methanol and hexane extracts of *O. basilicum* showed antibacterial activities against *Acinetobacter*, *Bacillus*, *Brucella*, *Escherichia*, *Micrococcus*, and *Staphylococcus*. Sanni et al. (2008) outlined that the extracts of *O. basilicum* were found to possess *in vitro* antibiotic activity against *S. aureus*, *Streptococcus* spp., *Salmonella* spp., *Shigella* spp. and *P. aeruginosa* at high doses. *C. albicans* was resistant to petroleum ether, chloroform and ethyl acetate extracts (Table 4). These results are in agreement with Kaya et al. (2008) who reported that *C. albicans* 845981, *C. crusei* ATCC 6258 and *C. albicans* 90028 were resistant to chloroform, acetone and methanol extracts of *O. basilicum* leaves, and in disagreement with Adigozel et al. (2005) who concluded that methanol and hexane extracts of *O. basilicum* aerial parts showed anticandidal activity against *C. albicans*. The antimicrobial activities of plants have been attributed to bioactive components such as alkaloids, saponins, tannins, flavonoids, steroids and anthraquinones (Chioma et al., 2014).

**Table 1:-** Phytochemical constituents of *Ocimum basilicum* leaf extracts.

Phytochemicals	Extract			
	Ethanol	Chloroform	Ethyl acetate	n-butanol
Flavonoids	+	+	-	+
Tannins	++	+	-	+
Terpenoids	++	++	+	+
Steroids	++	++	+	+
Saponins	++	+	++	+++
Reducing sugars	+	+	++	+

Key: +: Presence; ++: Present; +++; Present; -: Absence

**Table 2:-** Total phenolic content (mg GAE/gm) of *Ocimum basilicum* leaves extracted with different solvents.

Extract	Total phenolic content (mg GAE/gm)
Ethanol	166.03±38.83 <sup>a</sup>
Petroleum ether	182.90±107.80 <sup>a</sup>
Chloroform	171.67±16.91 <sup>a</sup>
Ethyl acetate	106.33±57.90 <sup>a</sup>

The values are presented as the mean ± the standard deviation (n = 3).

Mean values with similar letters within a column are not significantly different (p>0.05).

**Table 3:-** DPPH radical scavenging activity (%) of *Ocimum basilicum* leaves extracted with different solvents.

Extract	Antioxidant activity (%)
Ethanol	29.93±9.51 <sup>a</sup>
Petroleum ether	32.05±7.97 <sup>a</sup>
Chloroform	44.10±10.28 <sup>a</sup>
Ethyl acetate	46.00±6.85 <sup>a</sup>

The values are presented as the mean ± the standard deviation (n = 3).

Mean values with similar letters within a column are not significantly different (p>0.05).

**Table 4:-** Antimicrobial activity (inhibition zone in mm) of *Ocimum basilicum* leaf extracts determined by agar disc diffusion assay (100 mg/ml).

Microorganisms	Extract				S.L.
	Ethanol	Petroleum ether	Chloroform	Ethyl acetate	
<b>Gram-positive</b>					
<i>Escherichia coli</i>	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
<i>Pseudomonas aeruginosa</i>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	10.00±0.00 <sup>a</sup>	0.00±0.00 <sup>b</sup>	***
<b>Gram-negative</b>					

<i>Staphylococcus aureus</i>	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
<i>Bacillus cereus</i>	0.00±0.00 <sup>c</sup>	10.00±0.00 <sup>b</sup>	13.00±0.00 <sup>a</sup>	0.00±0.00 <sup>c</sup>	***
<b>Fungus</b>					
<i>Candida albicans</i>	10.00±0.00 <sup>a</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>b</sup>	***

The data are presented as mean ±standard deviation (n=3)

Means in each row bearing similar superscripts are not significantly different (P>0.05)

\*\*\* = P<0.001

S.L. = Significance level

### Conclusion:-

*O. basilicum* leaves extracted with ethanol, chloroform, ethyl acetate and n-butanol contain flavonoids, tannins, terpenoids, steroids, saponins and reducing sugars. Ethanol, petroleum ether, chloroform and ethyl acetate extracts reported to have total phenolic compounds and antioxidant activity. *E. coli* and *S. aureus* were resistant to ethanol, petroleum ether, chloroform and ethyl acetate leaf extracts, while these extracts had varying degrees of activity against *P. aeruginosa*, *B. cereus* and *C. albicans*.

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