

RESEARCH PAPER

ANTIMICROBIAL, ANTIOXIDANT AND ANTICANCER SCREENING OF *OCIMUM BASILICUM* SEEDS

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Basil seeds are traditionally believed to be used for therapeutic purpose to improve blood circulation, reduce inflammation, reduce the oxidation of cholesterol, and increase immune function and to control blood sugar level. In present study, basil (*Ocimum basilicum*) seeds were used as the raw material for evaluation of their bioactive compounds. Active components of the seeds were extracted using Soxhlet apparatus with two different solvents petroleum ether and methanol. Basil seeds extract exhibited strong antibacterial activity against nine pathogenic bacteria. The strongest inhibitory activity of basil seeds extract was observed against *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella dysenteriae* and *Klebsiella pneumoniae*. As per DPPH assay, the maximum free radical scavenging activity (73.85%) was shown by petroleum ether extract and the minimum activity (34.20%) was shown by methanol extract of basil seeds. Moreover, anticancer activity results clearly indicated that the basil seeds are a potential source of stable bioactive compounds.

Key words: Basil seeds, Antioxidant activity, DPPH assay, Antimicrobial activity.

INTRODUCTION

Since decades, plants and their parts are well known for their bioactive constituents responsible for the therapeutic effects (Jain *et al* 2011; Agarwal *et al* 2015; Shrestha *et al* 2016). Basil, also called as 'king of herbs', contains plenty of phytochemicals with significant nutritional as well as antioxidant capabilities and health benefits. Basil seeds are authenticated as *Ocimum basilicum* Linn, belonging to family 'Lamiaceae' which is an annual plant usually producing white-purple flowers (Daneshian *et al* 2009). It is a culinary herb consumed in high quantity due to the characteristic flavors it imparts (Naghbi *et al* 2005). This plant is found in many parts of the world especially in the tropical regions of Asia, Africa and Central and South America (Paton *et al* 1999). Basil seed is black in color and oval in

shape with mean dimensions of 3.11 ± 0.29 mm (length), 1.82 ± 0.26 mm (width) and 1.34 ± 0.19 mm (height). *Ocimum basilicum* dried seeds and water soaked seeds are presented in **Figure 1**.

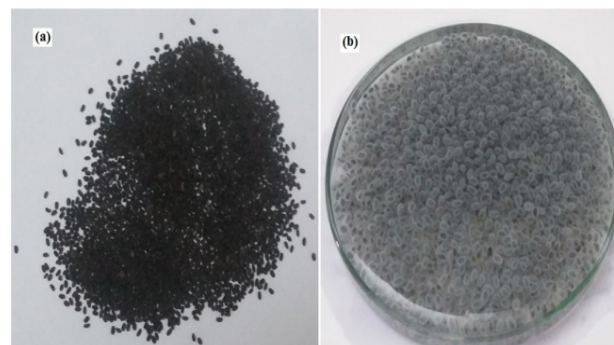


Fig. 1. *Ocimum basilicum* seeds (a) dried seeds (b) seeds soaked in water

Basil seeds have been used in traditional medicine for a long time to treat colic ulcer, dyspepsia, diarrhoea and inflammations, among others ailments. In many parts of Asia, basil seeds are frequently included in beverages (Sharbat) and ice desserts (Faloodeh) for aesthetic purposes as well as a source of dietary fibre. Sweet basil seeds are also known as sabja seeds, falooda seeds, tukamaria seeds. Many herb spices, especially those belonging to the Lamiaceae family, such as sage, oregano, and thyme, show strong antioxidant activity (Hirasa and Takemasa, 1998). *Ocimum* genus contains between 50 to 150 species of herbs and shrubs from the tropical regions of Asia (Bailey, 1924). A number of phenolic compounds with strong antioxidant activity have been identified in these plant extracts. When the seed of *O. basilicum* L. is soaked in water, the outer pericarp swells into a gelatinous mass (Azuma and Sakamoto, 2003) due to the presence of a polysaccharide layer. Polysaccharides are usually used as gelling, thickening and stabilizing agents to improve stability and textural properties of many food products such as jellies, salad dressings and desserts. The mucilaginous layer of the swollen seeds is a pectinous matrix, consisting of considerable amounts of unesterified galacturonic acid with a large capacity for hydration (Fahn and Werker, 1972). *Ocimum basilicum* seeds contain a reasonable amount of hemicellulose and cellulose, accounting for their hydrophilic character. They are high in fibre and associated nutritional properties, and can be considered as a new non-conventional source of fibre (Mathews *et al* 1993). Basil seeds possesses generous percentage of carbohydrate (42%), fats (25%) and proteins (20%) and are an magnificent source of fibre (in only 4 grams of basil a seed there are more dietary fiber than a whole bulb of lettuce), alpha-linolenic acid (ALA) which comes from the high level of omega-3 fatty acid present in the seeds. It contains zero percent calories, antibacterial, anti-spasmodic, antiviral, carminative and nervine. The seeds are used to reduce body heat and nervous debility. Superiority of using seeds rather than leaves in growth performance as the seeds contain much higher content of protein and lipid relative to leaves and also basil seeds contain active compounds such as planteose, mucilage, polysaccharides and fixed oil that consists of linoleic acid (50%), linolenic acid (22%), oleic acid (15%) as well as 8% unsaturated fatty acids (List and Horhammer 1977). The objective of the

present study was to evaluate the antibacterial, antioxidant, and anticancer activities of *O. basilicum* seeds extract and explore its use in pharmaceuticals excipients.

MATERIALS AND METHODS

Chemicals

The *O. basilicum* seeds (locally known as Sabja) used in this study, were procured from the local market, Vellore, Tamilnadu. Petroleum ether, methanol was purchased from Himedia Pvt. Ltd. Mumbai. DPPH (1, 1-Diphenyl-2-picrylhydrazyl) were obtained from Sigma Chemicals (St. Louis, Missouri, USA). All other solvents and reagents used were of analytical grade.

Soxhlet extraction

The *O. basilicum* seeds were uniformly grinded using mechanical grinder to make fine powder. The powder was serially extracted in petroleum ether and methanol, separately using a Soxhlet apparatus. The cycles of petroleum ether and methanol were run till complete defatting was obtained. The crude solvent extracts of *O. basilicum* seeds was then dried at room temperature and stored.

Phytochemical screening

The filtered crude extract of *O. basilicum* seeds was used for screening of phytochemical qualitative reactions such as carbohydrates, alkaloids, flavonoids, saponins, tannins and phenolic acids. The colour intensity of the precipitate which was formed was used as analytical test controls.

Test for carbohydrates (Fehling's test)

About 1 ml of Fehling A and Fehling B solution was added to the extract. Then, it was allowed to heat for 30 min and observed for the formation of brick red color which indicated the presence of carbohydrates.

Test for alkaloids (Wagner's test)

A small amount of extract was taken in a test tube and 3-5 drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water) was added. Formation of reddish brown precipitate or coloration indicated the presence of alkaloids in the extract.

Test for saponins (Foam test)

About 0.1 g of sample was mixed with 5 ml of distilled water and allowed to boil. Then the mixture was filtered and 2 drops of olive oil was

added in 1ml of filtrate. The mixture was shaken and formation of emulsion and froth was observed. The 1ml filtrate was diluted by adding distilled water up to 4 ml. The mixture was shaken vigorously and observed for a stable froth.

Test for flavonoids

The filtrate was prepared by boiling the mixture of 0.5 g of sample and 10 ml of ethyl acetate for 1 min. Then, the mixture was filtered and 4 ml of filtrate was shaken with 1 ml of 1% ammonium chloride solution. Formation of yellow color in the presence of ammonium solution indicated the presence of flavonoids.

Test for phenols (Ferric chloride test)

About 1 ml of extract was mixed with 1 ml of distilled water and warmed. To this, 2 ml of ferric chloride solution was added. Formation of green or blue color confirmed the presence of phenols.

Test for tannins

About 5 g of the dried and powdered sample was boiled in 20 ml of water in a test tube with the aid of a water bath and was filtered. Then, few drops of ferric chloride was added. Appearance of brownish green or bluish black coloration indicated the presence of tannins.

In-vitro antioxidant assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals scavenging activity

To check the antioxidant activity through free radical scavenging activity by *O. basilicum* seeds, the change in optical density of DPPH radicals was monitored.

The *O. basilicum* seeds extracts at 1 mg/ml concentration was used. DPPH is a stable, nitrogen-centered free radical which produces violet color in ethanol solution. When a substrate that can donate a hydrogen atom added in DPPH solution, it was reduced to a yellow colored product, diphenylpicryl hydrazine. DPPH solution (0.5 mol/l) was prepared in 95% methanol. The sample extracts (0.2 ml) was diluted with methanol. A total of 2 ml of DPPH solution (0.5 mol/l) was added to the test samples and incubated for 30 min at room temperature in darkness. After 30 min, the absorbance was measured at 517 nm using UV-spectrophotometer (Nicolet, Evlution-300, Germany). Free radical scavenging activity was expressed as a percentage.

The percentage of the DPPH radical scavenging was calculated as:

$$\text{Antioxidant activity (\%)} = \left[\frac{\text{Control absorbance} - \text{Extract absorbance}}{\text{Control absorbance}} \right] \times 100$$

Antibacterial study

In vitro antimicrobial studies were carried out on nine bacterial strains such as *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus* sp, *Proteus mirabilis*, *Shigella dysenteriae*, *Salmonella* sp, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Pseudomonas aeruginosa* by well diffusion method. The clinical pathogenic strains were obtained from Microbial Biotechnology Lab, VIT University. Four wells were aseptically punctured by using sterile borer and different concentrations (25, 50, 75 and 100 mg/ml) of sample extracts were loaded into the wells. The plates were incubated at 37°C for 24 h and zone of inhibition was measured around the wells.

Anticancer activity

Cell line

The human osteosarcoma cell lines (MG63) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37 °C, 5% CO₂, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and culture medium was changed twice a week.

MTT assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyl tetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan.

Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15 μ l of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37 °C for 4 h. The medium with MTT was then flicked off and formazan crystals was formed which was solubilized in 100 μ l of DMSO and then absorbance was measured at 570 nm using micro plate reader. The percentage cell viability was then calculated with respect to control as follows:

$$\text{Percentage of cell viability} = \left[\frac{\text{[A] Test}}{\text{[A] Control}} \right] \times 100$$

The percentage of cell inhibition was determined using the following formula:

$$\text{Percentage of cell inhibition} = 100 - \frac{\text{Abs (sample)}}{\text{Abs (control)}} \times 100$$

Analytical methods

Thin layer chromatography

TLC was done to separate the compounds of *O. basilicum* seeds extracts. Combination of solvents (hexane: acetone; 60:40) was used as mobile phase to separate the compounds according to their mobility. Separated spots were visualized under UV light at 254 nm and R_f factor was calculated by the following equation.

$$R_f \text{ factor} = \frac{\text{Distance travelled by solute front}}{\text{Distance travelled by solvent front}}$$

Gas chromatography mass spectrometry (GCMS)

GC-MS was used to identify the bioactive compound of *O. basilicum* seeds. Perkin Elmer Clarus 680 gas chromatographic instrument equipped with a mass spectrometer detector (Clarus 600 model) and an Elite-5MS (30.0 m, 0.25 mmID, 250 μ m df) column was used. The carrier gas used was helium at a flow rate of 1ml/min. The following temperature program was used: initially the oven temperature was held at 60°C for 2 min and then ramped from 10°C/min to 300°C with hold time for 4 min, total run time 30 min. The temperature of the injector was maintained at 300°C. The ion trap was operated at 70 eV in scan mode (50-600 m/z). A sample of 1 μ l was injected in split mode (10:1). As each chemical passes through the MS, characteristic molecular fragments are produced permitting identification based on the Wiley registry of mass spectral data.

RESULTS AND DISCUSSION

Phytochemical study revealed the presence of different phytochemical constituents in petroleum ether extracts of *O. basilicum* seeds. The results of phytochemical analysis are presented in **Table 1**.

It confirms the presence of alkaloids, flavonoids, carbohydrates, tannins, terpenoids in petroleum ether extract of *O. basilicum* seeds. These phytochemicals are reported to possess different biological activities. *e.g.* Saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects (Manach *et al* 1996). Flavonoids, tannins and alkaloids have hypo-

Table 1. Qualitative analysis of phytochemical studies of petroleum ether extract of *Ocimum basilicum* seeds

Phytochemical test	Results
Alkaloids	
Hager's test	+
Wagner's test	-
Carbohydrates	
Fehling's test	+
Molish test	+
Phytosterols	
Liebermann Burchard's test	-
Phenols	
Ferric chloride test	-
Flavonoids	
Alkaline reagent test	+
Lead acetate test	
Tannin test	+
Terpenoids test	+
Salkowski test	-

glycemic activities (Oliver Bever, 1980; Cherian and Augusti, 1995). Terpenoids have the analgesic properties. The results obtained coincides with the previous studies conducted on Lamiaceous plants *Mentha piperita*, *Melissa officinalis* and *Rosmarinus officinalis* (Zheng and Wang, 2001), showing equivalent or higher antioxidant activity.

Analytical studies

TLC

Thin layer chromatography was done to separate the components present in petroleum ether extract of *O. basilicum* seeds by its characteristics R_f values. Two separate spots were observed under UV light using chloroform: methanol (8:2) as mobile phase. The spots on TLC plates indicate the presence of different components which were further analyzed by GC-MS.

GC-MS

In order to determine the compounds present in the extract of *O. basilicum* seeds, GC-MS analysis was done. GC-MS chromatogram of petroleum ether extract of *O. basilicum* seeds are presented in **Figure 2**. This analysis revealed that petroleum ether extract of *O. basilicum* seed contains different compounds. Some of them are known for their biological activity whereas, activity of few compounds remains unknown. From petroleum extract, gamolenic acid, eicosatrienoic acids, nonanoic acid are reported to possess antimicrobial activity (Valvi *et al*

2014). Nonanoic acid is reported to possess antimicrobial and antioxidant activity (Kumar *et al* 2010).

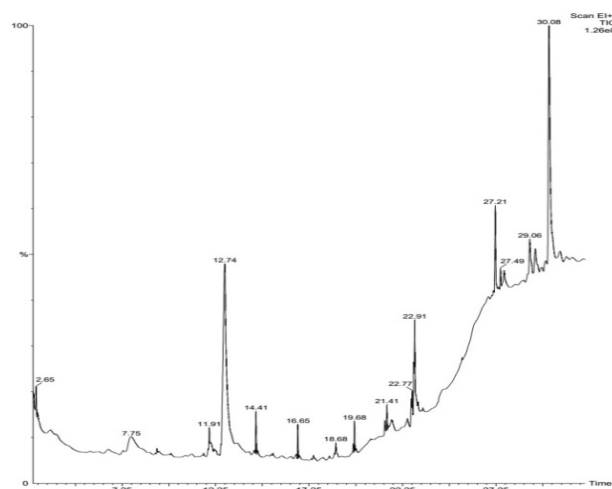


Fig. 2. GC-MS chromatogram of petroleum ether extract of *O. basilicum* seeds

Antimicrobial study

Antimicrobial activity of petroleum ether extract

of *O. basilicum* seeds was checked against nine clinical pathogens. Antimicrobial activity was determined by measuring zone of inhibition formed after incubation period. The petroleum ether extract of *O. basilicum* seeds showed characteristic zone of inhibition against all pathogens. Highest zone of inhibition was observed at 100 mg/ml concentration against *Pseudomonas aeruginosa*. Petroleum ether extract of *O. basilicum* seeds showed antimicrobial activity against both Gram-positive and Gram-negative bacteria (Adamu *et al* 2005). **Table 2** presents the result of the antimicrobial activities of *O. basilicum* seeds extracts.

Antioxidant activity

The antioxidant activity of the extracts was evaluated by DPPH radical scavenging assay. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a synthetic free radical with deep violet color when is in form of solution which has a λ max at 517 nm. Petroleum ether extract of *O. basilicum* seeds has shown higher antioxidant activity than methanol extract (**Table 3**).

Table 2. The diameter of zone of inhibition of *Ocimum basilicum* seeds against clinical pathogens

Clinical pathogens	Zone of inhibition (mm)			
	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
<i>Staphylococcus aureus</i>	-	6	8	11
<i>Escherichia coli</i>	11	-	-	-
<i>Salmonella sp.</i>	-	9	10	-
<i>Shigella dysenteriae</i>	-	14	14	14
<i>Klebsiella pneumonia</i>	-	8	13	-
<i>Serratia marcescens</i>	-	6	4	-
<i>Enterobacter sp.</i>	6	9	15	-
<i>Pseudomonas aeruginosa</i>	-	-	16	-
<i>Proteus mirabilis</i>	-	10	12	-

Table 3. Antioxidant activity of *Ocimum basilicum* seeds

<i>Ocimum basilicum</i> seeds	OD at 517 nm	% Scavenging activity
Standard (10 mg/ml)	1.902	-
Petroleum ether	0.603	73.85
Methanol extract	1.71	34.20

Increased absorbance of reaction mixture indicates increased reducing power of the extract. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. This result suggested that 73.85% of the antioxidant capacity of *O. basilicum* seeds results from the contribution of phenolic compounds. Also, it can be concluded

that antioxidant activity of seed extracts is not limited to phenolics. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They may also have a metal chelating potential (Rice-Evans *et al* 1995).

Anticancer activity

Research into food-derived bioactive components for cancer prevention as well as

cancer therapy is a growing area of research due to the relatively low or no detectable toxicity and better bioavailability. *O. basilicum* seeds extracts were evaluated for cytotoxic activity on human osteosarcoma cell lines (MG63). It showed cytotoxic effect on MG63 human osteosarcoma cell lines. MTT cell growth inhibition assay was taken as *in vitro* measure of anticancer activity of *O. basilicum* seeds by using MG63 human osteosarcoma cell lines. The cell viability percentage showed maximum activity at the lower concentration *i.e.* 12.5 µg/ml (Figure 3). There was more death of cell line or cell deterioration with increase of concentration of *O. basilicum* seeds. There was an enlargement of the cells observed at high concentration of *O. basilicum* seeds and the cells became shrunken with membrane blebbing and showed signs of detachment from the surface of the wells denoting cell death.

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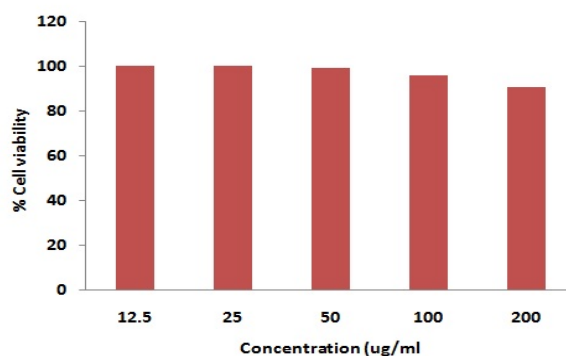


Fig. 3. Anticancer activity of *Ocimum basilicum* seeds

CONCLUSION

From the above study, *O. basilicum* seeds are proven to have good antimicrobial, antioxidant and anticancer activities. Bioactivity of *O. basilicum* seeds has projected the great importance of functional foods.

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