IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
September 2016 Vol.:7, Issue:2

Phytochemical and Biological Evaluation of *Ocimum basilicum* Roots

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
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ISSN 2349-7203
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Submission: 5 September 2016
Accepted: 10 September 2016
Published: 25 September 2016

Keywords: *Ocimum basilicum* root, phytochemical, antibacterial, antioxidant, α-glucosidase, carbonic anhydrase

ABSTRACT

Ocimum basilicum Linn. is an incredible plant with analgesic, anti-inflammatory, antimicrobial, antioxidant and hepatoprotective property. In the present study, roots of *Ocimum basilicum* were investigated for phytochemical and biological activities simultaneously. Phytochemical screening of plant roots endorses the presence of flavonoids and terpenes. The crude root extract in DCM possesses moderate antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aureginosa* whereas, methanol extract reveals significant antioxidant capability in DPPH radical scavenging activity(IC_{50}value29.64µM).The enzyme inhibitory potential of *Ocimum basilicum* root showed strong *carbonic anhydrase inhibition* (79%±0.09) by DCM extract and methanol extract possess robust (94%±0.18) *α-glucosidase* inhibitory potential.

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1 INTRODUCTION

The traditionalpractice of medicinal plants implies in ethnobotanical literature. World Health Organization (WHO) reports 80% of the world depends on plant extracts and infusions as folk medicine [1]. People in developing countries of Asia and Africa relies chiefly on natural herbs and plants for health management[2]. According to *Mohantay et al.* less than 10% of ethnic medicinal plants [3] have been scientifically analyzed for their potential bioactivity and toxicity profile[14].Nevertheless, contemporary health management systems also utilize 25% plant derived medicines [5] which comprise of aspirin from *Salix alba* and quinine from Cinchona tree bark [6].

Family Labiatae comprises of important medicine plant known for their cardiovascular, antioxidant, neurological and anticancer potential[7]. *Ocimum basilicum* belongs to Labiatae family reported to be effective against pathogenic microorganisms, inflammation, ulcer, diarrhea and hypoglycemic condition[8]. Studies on leaves of *Ocimum basilicum* leaves have reported possessing hepatoprotective [9]antimicrobial [10]antimalarial [11] chemopreventive and antioxidant potential. Furthermore, roots have been investigated for biological activities such as antibacterial [12], phytotoxic [13], antioxidant [14].

Ocimum basilicum is a rich source of secondary metabolites such as glycosides, proteins, amino acids, tannins, phenolic compounds, sterols, saponins[15], flavonoids [16] and terpenes [17]. Terpenes are found to exhibit antimicrobial, antiviral and anti-inflammatory activity [18]. While, Flavonoids exert anti-oxidative effects as free radical scavengers and possess therapeutic potential against edema, osteoporosis, obesity and even cancer [19]. Moreover, consumption of phenolic compounds reduces the risk of coronary heart disease (Okawa *et al.,* 2001) and *carbonic anhydrase-I* and *carbonic anhydrase-II inhibitory* effects which showantiobesity, antitumor and anti-diabetic potential[20].

Diabetes mellitus common metabolic disorder characterized by high plasma glucose levels and flavonoids are responsible for the decrease in plasma cholesterol levels in type-II diabetes [21]. Alpha-glucosidase inhibitors delay the uptake of carbohydrates leads to suppression of postprandial hyperglycemia [22].The present study evaluates *Ocimum basilicum* roots for phytochemical screening, antioxidant effect, antibacterial, *carbonic anhydrase and α-glucosidase* inhibition potential.

2 MATERIAL AND METHODS

Ocimum basilicum roots were collected from Multan, identified (voucher specimen: Stewart 626-6) by Dr. Zafar Ullah Taxonomist, Bahauddin Zakariya University, Multan Pakistan.

2.1 Extraction and Phytochemical Analysis

Roots of *Ocimum basilicum* were shade-dried and then extracted with dichloromethane (OBRD) and methanol (OBRM). For preliminary phytochemical analysis, *Ocimum basilicum* roots (Table:1) were subjected to qualitative analysis for alkaloids, anthraquinones, cardiac glycosides, saponins, flavonoids, and terpenes[16],[17], [23].

Alkaloids

(a) Dragendorff's Reagent: Potassium bismuth iodide solution indicates the presence of alkaloid by reddish brown precipitation. (b) Mayer's Reagent: Potassium mercuric iodide solution produces cream color precipitate indicating the presence of alkaloids. (c) Wagner's Reagent: Potassium Iodide in Iodine solution gives reddish brown precipitate to confirm the presence of alkaloids.

Cardiac Glycosides

(a) Keller-Kilianitest: To glacial acetic acid a few drops of ferric chloride was added and then shifted to test tube with concentrated sulphuric acid. Blue coloration indicates the presence of glycosides.

Flavonoids

(a) Ferric chloride test: To plant extract added few drops of ferric chloride, strong green color shows the presence of flavonoids. (b) Alkaline reagent: Alkaline reagent with tiny sodium hydroxide strong yellow color appears which become colorless by acetic acid (dilute) confirms the presence of flavonoids.

Triterpenoids

(a) Salkowski's test: To plant extract add sulphuric acid (concentrated), the appearance of red colouration in the lower layer of test tube indicates the presence of steroids whereas, the lower layer of yellow color shows the presence of triterpenoids. (b) Libermann Burchard's test: To plant extract add traces of acetic anhydride, after boiling, the addition of sulphuric

acid gives a brown ring, green coloration of upper layer indicates steroids whereas, red colouration shows the presence of triterpenoids.

Saponins

Frothing test: Plant extract solution in distilled water on vigorous shaking, the appearance of foam confirms saponins.

2.2 Biological Evaluation

2.2.1 Antibacterial Activity

Microplate alamar Blue antibacterial assay was performed on [12]. *Escherichia coli* (ATCC#2592), *Shigella flexenari* (ATCC#12022), *Staphylococcus aureus* (NCTC#6571) and *Pseudomonas aureginosa* (NCTC#10662). Middle-brook Supplement i.e. 7H9-S was used as growth medium. 7H9-Supplement containing alamar blue dye was used as negative control whereas bacterial suspension diluted with antibiotic ofloxacin solution was referred as a negative control. Microplates were incubated for specified duration at 37^{o}C. After specified time period color changes and minimum inhibitory concentrations were recorded (Table: 2).

2.2.2 Antioxidant Activity

Antioxidant potential of the crude extract was evaluated by DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity [14].Extract in 5µl concentration was added to 95µl of DPPH and prepared a reaction mixture. The reaction mixture was added to 96-well plates and incubated for 30min at 37°C after incubation absorbance was taken at 515nm. Radical scavenging activity (RSA) was calculated by decolorization of DPPH with reference to standards, gallic acid and N-acetylcysteine (Fig.1).

2.3 Enzymatic Activities

2.3.1 *Carbonic anhydrase* inhibition activity

Carbonic anhydrase inhibition assay was accomplished by (Ashiq *et al*., 2015) buffer (140µl) of pH 7.4 (20Mm HEPES), enzyme 20µl (C2624, PCode: 1001584424)0.1-0.2mg/ml of deionized water, and plant extract 20µl (0.5mg/ml in DMSO) incubated at 25^{o}C, after15min absorbance was documented at 400nm. To this incubated mixture substrate 4-nitrophenol acetate (20µl) (N8130, lot#BCBK4587V) was added and incubated at 25^{o}C,

after30minabsorbance was taken at 400nm. Acetazolamide was used as reaction control and % inhibition was calculated (Table: 3).

2.3.2 *α glucosidase* inhibition activity

To evaluate the α-glucosidase inhibitory[24] potential of extract, 70µl of buffer were added to 96 well plate and incubated at 30^{o}C. After 15min absorbance was noted at 400nm. The substratein 10µl concentration was added to the mixture and again incubated at 30^{o}C. After 30min absorbance was measured at 400nm, acarbose was used asacontrol. IC_{50} (µg/ml) values were calculated (Table: 3).

3. RESULTS ANDDISCUSSION

Ocimum basilicum is a natural reservoir for the treatment of cardiovascular, diabetes[25]bacterial [26]and viral disorders[27]. Since phytochemicals are prominent target drugs, their screening is preliminary step to identify the diversity and biological property. Several reports on phytoconstituents of *Ocimum basilicum* confirms the presence of alkaloids, triterpenoids, phenyl propanoids, polyphenols, flavonoids, oleanolic acid and saponins[28]. In this study, qualitative screening of secondary metabolites in *Ocimum basilicum* roots reveals the presence of flavonoids and terpenes (Table:1). Essential oils of *O. basilicum* has shown significant antibacterial potential against gram-negative bacteria. Concerning prior reports, *Ocimum basilicum* leaves were found active against *Salmonella aureus, Salmonella paratyphi, Salmonella typhi, Salmonella vulgaris* and *E. coli*[29]. Our study shows *Ocimum basilicum* root (DCM) possess moderate antibacterial potential *Staphylococcus aureus* and *Pseudomonas aureginosa* in microplate almar Blue assay using ofloxacin as a standard antibacterial drug (Table: 2). Moreover, flavonoids are considered to be involved in antioxidant, anti-inflammatory[30]. Similarly, our results concords with previous [31] and methanol extract OBRM has produced significant antioxidant potential with IC_{50} 29.64µM90 and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was asses as 625% RSA (Fig.1). Besides this, the result of radical scavenger activity (RSA) indicates the extraordinary hydrogen donation ability of ORBM (90.6%). *Ocimum basilicum* roots were examined for *carbonic anhydrase and α-glucosidase* enzyme inhibition to identify novel, nontoxic drug candidate with antitumor, anti-diabetic potential (Fig. 2). The carbonic anhydrase enzyme serves as a catalyst during reversible hydration of carbon dioxide to bicarbonate corresponds to breathing and carbon dioxide homeostasis [32]. Carbonic

anhydrase inhibitors are clinically used as a drug against diabetes and tumorogenesis[33]. *Ocimum basilicum* root extract OBRD exhibited 79±0.09% inhibition of *carbonic anhydrase*, same results were observed in an earlier study[19]. Moreover, *α-glucosidase* enzyme hydrolyzes terminal non-reducing α-glucose which leads to raising in blood glucose level[34]. Currently, acarbose is used as *α-glucosidase* inhibitor drug although reported to have adverse effects and drug resistance[35]. Our result shows methanol extract OBRM possess remarkable *α-glucosidase* inhibition 94%±0.18and OBRD moderate inhibition potential 60%±0.12 as compare to acarbose the standard drug.

4 CONCLUSION

Ocimum basilicum is a prominent medicinal plant, its phytochemicals are considered as ideal drug candidate against various diseases. Preliminary studies have found valuable secondary metabolites as constituents of *Ocimum basilicum* which are found to be effective against pathogenic bacteria and possess significant antioxidant effect. Results reveal *Ocimum basilicum* also have *carbonic anhydrase* and *α-glucosidase* inhibition potential and can be robust against tumorigenesis and Diabetes. Present results, encourage further extensive studies leading to isolation and purification of bioactive compounds.

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Tables

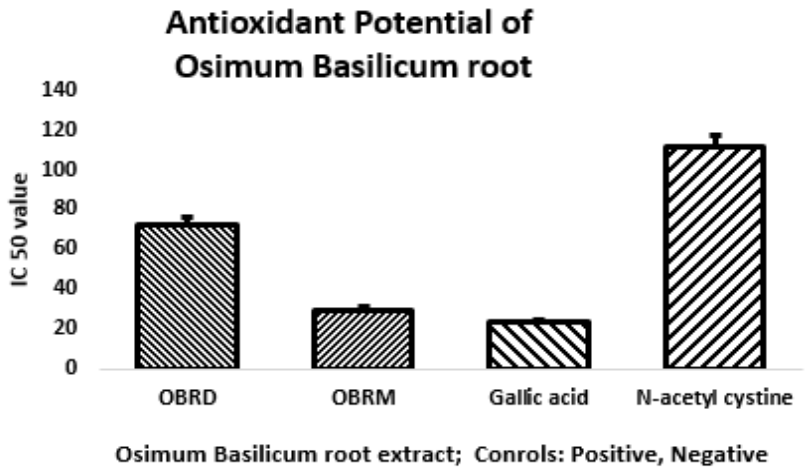
(Table: 1) Preliminary evaluation of secondary metabolites in *Ocimum basilicum* root

Secondary Metabolites	**Result**
Alkaloids	Negative
Cardiac Glycosides	Negative
Flavonoids	Positive
Terpenes	Positive
Saponins	Negative

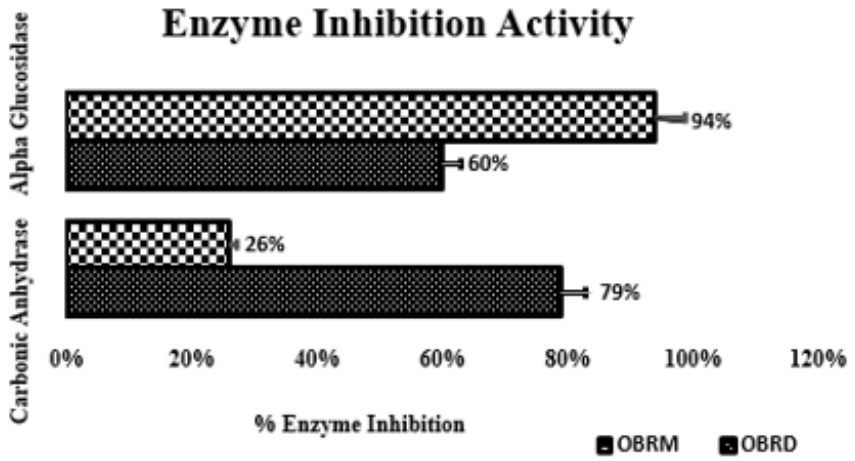
(Table: 2) Microplate Alamar Blue Assay for antimicrobial activity of *Ocimum basilicum* root extract (DCM)

Microplate Alamar Blue Assay	**% Inhibition**	
Micro-organisms	**OBRD Extract**	**Std. Drug (Ofloxacin)**
Escherichia coli	-	86.735%
Shigella flexenari	0.796%	82.147%
Staphylococcus aureus	57.8%	81.377%
Pseudomonas aureginosa	37.9%	84.536%

Figures



(Figure: 1) DPPH, Radical Scavenging Potential of OBRD (47.463%) and OBRM (90.625%) extracts



(Figure: 2) *Carbonic anhydrase* and *α-Glucosidase* inhibition