Advances in Environmental Biology, 10(7) July 2016, Pages: 84-89

AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/

Phytochemical Composition, Antibacterial and Antifungal Activities of Sweet Basil (*Ocimum basilicum)*

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Received 12 June 2016; Accepted 28 July 2016; Available online 25 August 2016

ABSTRACT

The emergence of the synthetic drug has paved way for researchers to explore for alternatives, going to the basic source of drugs. Plants have been used for their healing capabilities because it was proven to cure ailments since ancient times. This research was carried out to evaluate the phytochemical composition of *Ocimum basilicum* (sweet basil) and to test its antibacterial and antifungal activities. Aqueous extraction of the leaf samples was done by soaking 50g of the powdered leaves on one liter of distilled water for 24 hours and following the protocols of Sofowara (1993) for the qualitative phytochemical screening. Results showed that eight phytochemicals in moderate and high amounts were present on the aqueous extracts of the sweet basil namely Alkaloids, Saponins, Flavonoids, Tannins, Glycosides, Steroids, Terpenoids and Resins. Antibacterial activity showed that after 24 hours of incubation, extracts with 10µl, 100µl and 1000µl do not differ statistically against *E.coli*. On the other hand, 1000µl of sweet basil aqueous extracts against *S. aureus* is comparable to the control (streptomycin) which suggests that extracts at this level of concentration could eradicate such pathogenic bacteria. Interestingly, antifungal activities of the aqueous extracts of sweet basil showed that 10mg/ml concentration could inhibit the growth of *Fusarium oxysporum*, a fungi known to cause wilts on crops. From the results, sweet basil leaf extracts can be useful in the field of pharmaceutical sciences as antibacterial agent and crop protection for its antifungal properties. The presence of its phytochemicals could mean that hydroponically grown sweet basil could further be studied for its mineral composition.

KEYWORDS: Basil, phytochemicals, E.coli, S. aureus, Fusarium

INTRODUCTION

Basil is the most popular annual herb in the world includes over 150 different species distributed in tropical regions of Asia, Africa, Central and South America [21]. Sweet basil extracts are used as flavouring and seasoning in foods and beverages as well as therapeutically for centuries [6][9]. There are more than 65 species of the genus *Ocimum*, basil is the major essential oil crop which is cultivated commercially in many countries [17]. It has a characteristic odor and sharp taste which probably originated from Asia and now being cultivated worldwide [8]. In recent years increased scientific interest in plant phytochemicals (plant chemicals) has brought numerous vegetables, herbs and spices – including basil – to the forefront of nutritional research [3]. Although the study of plant compounds is not new, scientists are only now beginning to characterize the wide range of

biologically active components in our food plants and investigate their impact on human health and disease. In cell culture and animal studies basil has been found to exhibit antimicrobial, anti-inflammatory, anti-diabetic, antioxidant and anti-cancer activity [8].

In the oriental regions, Tulsi (*Ocimum sanctum*) known as the Queen of Herbs. It is one of the most cherished of the many healing and health-giving herbs distributed mainly in the oriental region [14] It is called by names like Rama Tulsi, Krishna Tulsi in Sanskrit and Holy Basil in English. The natural habitat of Tulsi varies from sea level to an altitude of 200 m. It is found growing naturally in moist soil nearly all over the globe [19]. In Nepal, Aryan people grow Tulsi as a religious plant in their homes, temples and their farms. They use Tulsi leaves in routine worship. Three main forms are generally recognized Rama tulsi with stems and leaves of green, Krishna tulsi with stems and sometimes also leaves of purple and Vana Tulsi which is unmodified from its wild form.

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain [21]. Immediate actions must be taken to lessen this problem aiming for an efficient antimicrobial drugs. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency [7]. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant known by their active substances such as phenolic compounds which are part of the essential oils and tannins [18]. Currently, data on the antimicrobial properties of several plants, so far has measured empirical concurrently with the increasing number of reports on pathogenic microorganisms resistant to antimicrobials. Products resulting from plants may potentially control microbial growth in varied situations and cases of disease treatment, numerous studies have aimed to describe the chemical composition of these plant antimicrobials and the mechanisms involved in microbial growth inhibition, either separately or associated with conventional antimicrobials [22].

Thus, this present study was carried out to evaluate the phytochemicals, test the antibacterial activity and antifungal activity of *Ocimum basilicum* (sweet basil) grown hyroponically. Generally, this study will update the current information available for the sweet basil under Philippine tropical conditions specifically to deliver comparisons on the phytochemicals present on other studies and under Philippine hydroponic conditions.

MATERIALS AND METHODS

Source of Test Organism:

Pure cultures *of Fusarium oxysporum* was obtained from the Laboratory of fungal collection of RM-CARES, Research and Extension, CLSU. Bacterial strains of *E.coli* and *S. aureus* was obtained from the bacterial culture collection of Department of Biological Sciences, CLSU.

Aqueous Extraction:

Fifty grams of the powdered dried leaves were soaked in a sterile distilled water for 24 hours at room temperature. Extracts were filtered using Wattmann's filter paper no. 01. Filtered extracts were subjected to hot bath for two (2) hours at 70°C.

Phytochemical Evaluation of Sweet Basil Extracts:

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal constituents under study were carried out in extracts using the standard procedures as described by Sofowara [24].

Alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered. Filtrates were treated with Wagner's reagent (Solution of Iodine in Potassium Iodide). Formation of a reddish brown colored precipitate indicates the presence of alkaloids.

Saponins:

Two (2) g of powdered sample of each sample is boiled together with 20ml of distilled water in a water bath and filtered. 10ml of the filtered sample is mixed with 5ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing is then mixed with 3 drops of olive oil and for the formation of emulsion which indicates the presence of saponins.

Flavonoids:

Two to three drops of 1% NH_3 solution is added to the aqueous extract of each sample in a test tube. A yellow coloration is observed if flavonoids compound are present.

Tannins:

0.5g of powdered sample of each plant is boiled in 20ml of distilled water in a test tube and filtered 0.1% $FeCl_3$ is added to the filtered samples and observed for brownish to green or a blue to black coloration which shows the presence of tannins. Green coloration indicates the presence of gallotannins while brown coloration indicates the presence of pseudotannins.

Glycosides:

One (1) ml of concentrated H_2SO_4 is prepared in test tube 5 ml of aqueous extract from each plant sample is mixed with 2ml of glacial CH_3CO_2H containing 1 drop of $FeCl_3$. The above mixture is carefully added to 1ml of concentrated H_2SO_4 so that the concentrated H_2SO_4 is underneath the mixture. If cardiac glycoside is present in the sample, a brown ring will appear indicating the presence of the cardiac glycoside constituent.

Antibacterial Bioassay:

Preparation of Culture Media and Pour Plating:

Thirty-eight grams (38g) of Prepared Mueller-Hinton Agar (MHA) was mixed with one (1) liter of distilled water. It was heated until homogenous mixture was obtained. Approximately 300 ml of the prepared medium was dispensed onto a clean Erlenmeyer Flasks, plugged with sterile cotton and wrapped with paper and sealed with a rubber band. The medium was sterilized for 15 minutes at 15lbs/in^2,121°C using autoclave. After sterilization, these were allowed to cool for several minutes until ready for pour plating. Approximately 15mL sterile nutrient agar was aseptically dispensed into sterile petri plates then allowed to cool and solidified prior to inoculation of bacterial samples.

Spread Plating:

Bacterial inoculums of *E.coli* and *S. aureus* were loaded to sterile petri plates using spread plating method. 150 ul of each inoculums were aseptically transferred using micropipette, stainless metal spreader was used to spread the inoculums onto the plates in a circular motion.

Disc Diffusion Method:

Antibacterial activities of *O. basilicum* (sweet basil) was done following the Kirby-Bauer Method aginst *E.coli* and *S. aureus.* Six (6) mm of sterile paper discs were soaked on the aqueous extracts for about 30 minutes to allow absorption of the extracts. The discs were aseptically inoculated onto the plates with the test organisms with labels. Plates were incubated for 24-hours at room temperature. Zones of Inhibition were measured using a Vernier caliper after 8, 16 and 24 hours of incubation.

Experimental Treatments:

Treatments were laid out with three (3) replicates each. Treatments used in the study were of varying concentrations from 10 µl, 100 µl, 1000 µl and a positive control, commercial streptomycin to compare the results of the extracts at different level of concentration.

Antifungal Bioassay:

Revival of the Fungal Pathogen:

Approximately 10mm mycelial block from *F. oxysporum* was transferred on a clean sterile plate containing sterilized Potato Dextrose Agar (PDA) to allow proliferation until fully ramified.

Antifungal Bioassay:

Two (2) ml of prepared basil extracts at different concentrations were aseptically poured on a sterile standard plate and approximately 15-20 ml of sterilized PDA was added, aseptically swirled on a clockwise and counter clock wise motion to homogenize mixtures. Mixture was then allowed to cool and solidified.

Inoculation of the Test Organism:

Each plates containing mixtures were aseptically inoculated with a 10-mm fungal disc. Inoculated standard plates were incubated on a room temperature (28-32°C) and growth was measured using calibrated Vernier caliper for every 24-hours for 5 days.

Statistical Analysis:

Analysis was laid out Completely Randomized Design (CRD) with three (3) replications per treatment combination. The results presented are the means ± standard deviation of three replicates. The recorded data were treated statistically using the one way analysis of variance (ANOVA). The means were compared by Least Significant Difference test at $p < 0.05$ using SPSS v.20.

Advances in Environmental Biology, 10(7) July 2016, Pages: 84-89

RESULTS AND DISCUSSION

Phytochemical Screening of Sweet Basil Aqueous Extracts:

Numerous researches have been carried out to show that natural antioxidants in plants are closely related to their bio-functionalities such as the reduction of chronic diseases and inhibition of pathogenic bacteria growth, which are often associated with the termination of free radical propagation in biological systems [10]. Phytochemicals are naturally occurring constituents of plants [12]. Presented in Table 1 are the phytochemicals present in the aqueous extracts of sweet basil (*Ocimum basilicum*).

Table 1: Results of the Phytochemical Screening on Sweet Basil Aqueous Extracts

Mycochemical Test	Aqueous Extracts
Alkaloids	+++
Saponins	++
Flavonoids	+++
Tannins	++
Glycosides	++
Steroids	++
Terpenoids	+++
Resins	++

Key: − (Absent), + (Low in abundance), ++ (Moderate in abundance), +++ (High in abundance)

The phytochemical composition of *O. basilicum* aqueous extracts has showed inhibitory effects on both antifungal and antibacterial bioassays. These interesting results may be explained by the presence of tannins, which are known antimicrobial agents that could inhibit the growth of microorganisms by precipitating out the microbial protein and thus depriving them of nutritional proteins needed for their growth and development [27]. The same results were also reported by Daniel *et al.*, [11] basil aqueous extracts to contain saponins, tanins and cardiac glycosides. Aside from phytochemicals, they also reported that basil collected from Nigeria contains high amount of Potassium (K), Calcium (Ca), and appreciable amounts of Sodium (Na) and Magnesium (Mg).

Saponins are commonly known as an anti-nutrient, but it is also hypothetical to be useful in human nutrition for the regulation of cholesterols. Its presence *O. basilicum*, therefore could suggests that the plant is of therapeutic value [5]. Tannins are mordant, bitter plant polyphenols that either bind and precipitate or shrink proteins. They are distributed all over the plant kingdom. Tannins have traditionally been also considered as anti-nutrient but it may be associated medicinally in antidiarrheal, hemostatic and anti-hemorrhoids compounds. The presence of tannins in plants could be considered to be of homoeopathic value for its potential antiviral, antibacterial and anti-parasitic effects [4].

Table 2: Antibacterial Activities of *O. basilicum* aqueous extracts against *E. coli* and *S. aureus*

Treatments	Zones of Inhibition (mm)					
	E.coli			*S. aureus*		
Aqueous Extracts	8 hours	16 hours	24 hours	8 hours	16 hours	24 hours
10µl	9.53^{c}	21.92^{b}	27.43^{b}	7.68^{c}	11.63^{c}	16.88^{a}
100 µl	10.15^{b}	22.66^{b}	29.65^{b}	9.94^{b}	16.62^{b}	26.41^{b}
1000 µl	10.29^{b}	22.77^{b}	29.80^{b}	13.05^{a}	20.20^{a}	33.00^{a}
Control	11.26^{a}	33.54^{a}	36.77^{a}	10.14^{b}	22.64^{a}	36.72^{a}

Note: Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance

Table 2 showed the antibacterial activities of *O. basilicum* aqueous extracts on *E. coli* and *S. aureus* using Disc Diffusion Method. Results exuded by the basil extracts revealed that after 24 hours of incubation, 1000 µl, 100 µl and 10 µl do not differ statistically on the zones of inhibition against *E. coli*. The basil extract had a positive results for tannins, terpenoids and high content of alkaloids.

On the other hand, it was observed that after 24 hours of incubation, 1000 µl is significantly comparable to the results exuded by the control against *S. aureus*. Earlier results revealed that essential oils of sweet basil have better results on *S. aureus* among other Gram-positive bacteria [28]. Presence of such phytochemical compounds can explain the mechanism of action of the basil extract. The presence of tannins in plants can cause negative effect on productivity, reduced nutrient availability, reduced digestibility, impaired digestive physiology and may be mucosal perturbations for those who will intake such plants [13].

Results of this study supported the research done by Prasad [20] in which the oil extract of *O. basilicum* collected from different geographical regions, had high effectiveness on the Gram-positive bacteria in comparison to the Gram-negative ones. On the other hand, Adam and Omer [1] revealed their study on the antimicrobial activities of *O. basilicum* which exhibited a potential antibacterial activity against *Klebsiella pneumoniae* that showed significant difference at 50µg/disc concentration compared with ciprofloxacin, erythromycin and gentamicin respectively. Looking into the volatile compounds found in the extracts of *O.*

basilicum, Adeola *et al*., [2] concluded that the volatile oil of *O. basilicum*, therefore, exhibited a wide range of antibacterial activity and showed a noncompetitive inhibition against the extracellular protease of *Salmonella typhimurium.*

Table 3: Mean diameter (mm) of mycelial growth of *Fusarium oxysporum* against different treatments.

Treatments	Days of Incubation (mm)				
	Day 1	Day 2	Day 3	Day 4	Day 5
1mg/ml	14.96[a]	22.72[a]	32.36[a]	51.04[a]	64.45[a]
5mg/ml	13.40[a]	19.24[b]	29.57[b]	41.18[b]	52.23[b]
10mg/ml	11.19[b]	14.72[c]	20.40[b]	29.43[c]	41.91[c]
Negative Control	14.36[a]	24.44[a]	31.91[a]	56.23[a]	65.48[a]

Note: Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance

Results on Table 3 shows the fungal inhibitory mechanisms of *O. basilicum* aqueous extracts against *Fusarium oxysporum*, wilt-causing fungi in agricultural crops. Lowest mean diameter of 41.91mm was observed on 10mg/ml which expresses the inhibitory mechanism of the extracts to control the growth of the fungi. However, 1mg/ml resulted to a mean diameter of 64.45mm which is comparable to the negative control (untreated) with a mean diameter of 65.48mm. With these results, it can be concluded that aqueous extracts of *O. basilicum* could inhibit growth of such fungi.

The phytochemicals present in the aqueous extracts of *O. basilicum* may explain the mechanism exhibited to the antibacterial and antifungal activites. The occurrence of terpenoids in plants cause cytotoxic effects, growth hormones and tumor promoters [29]. Early researches by Wen, *et al.* [25], specific plant terpenoids can possess potent antiviral activities against Severe Respiratory Syndrome Coronavirus (SARS-Corv). Plants containing alkaloids have high Nitrogen organic constituents which can be attributed to their ability to become poisonous and even addictive [26]. Wooley [26] even suggested that alkaloids (*i.e.* pyrrolizidine and indolizidine) cause serious toxicity and even death for horses, cattle and sheep that graze on such types of plants.

Interesting results were also observed on the study of Koba *et al*., [15] showing inhibitory effects of *O. gratissimum* where it elicited various dermatophytes, imperfect filamentous fungi and pathogenic yeasts. On the other hand, Kocic-Tanackova [16] reported that *O. basilicum* extracts have eliminated various species of *Penicillium* in different levels of concentration and concluded that basil leaf extracts could be of use in post-harvest storage of foods.

This research was primarily carried out for the evaluation of phytochemicals, antibacterial and antifungal properties of the plant sample. Based on the results, it can be concluded that the aqueous extracts of sweet basil contains eight of the primary phytochemicals including tannins, saponin, flavonoids, alkaloids, steroids, terpenoids, resins and glycosides which is essential for the antibacterial and antifungal activity of such plants. Also, because of these phytochemicals, sweet basil extracts could be useful in therapeutic medicine for its antibacterial activities against *E. coli* and *S. aureus*. On the other hand the antifungal activities of this plant against *F. oxysporum* could be useful for preventing wilt on crops and would help to improve post-harvest lost.

ACKNOWLEDGMENTS

The authors would like to give thanks to the CLSU Hydroponics Team under the supervision of Dr. Chito F. Sace and to the Department of Science and Technology (DOST) under Dr. Josette T. Biyo for funding the research. Also, high appreciation is given to Dr. Cynthia Cervero – Divina for inspiring the authors to do the preliminary study on hydroponically grown sweet basil.

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