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Screening of Antibacterial Potential of Siam Queen, Holy Basil and Italian Basil Essential Oils

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Basil (*Ocimum basilicum*) is a widespread culinary herbal crop grown for fresh or dry leaf, essential oil and seed markets. The essential oils of three basil cultivars were investigated for their antibacterial activity. The essential oil of Siam Queen, Holy basil and Italian basil was tested against two gram positive (*B. subtilis* and *M. lacticum*) and two gram negative (*Y. reukeri* and *X. axonopodis*) bacterial strains in comparison with antibiotic, amoxicillin. Highly significant differences were recorded for essential oils/antibiotic, concentration and bacteria. Mean values for zones of inhibition against different bacterial strains demonstrated that basil essential oils of all the studied cultivars have good antibacterial activity against gram positive bacteria (*B. subtilis*) as compared to gram negative bacteria.

Keyword: Basil, Antibacterial Activity, Essential Oil, Italian Basil, Siam Queen, Holy Basil.

1. 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 [1, 2]. Basil is included in the major essential oils producing species belonging to the genus Ocimum [3] and often referred as the "King of the herbs, being widely utilized due to its economic, nutritional, industrial and medicinal importance [4]. Basil extracts are used as flavoring & seasoning agents in foods and beverages as well as therapeutically for centuries [5, 6]. In folk medicine, basil is used due to its carminative, stimulant and antispasmodic properties. Basil has shown antioxidant, antimicrobial and antitumor activities due to its phenolic acids and aromatic compounds [7]. The medicinal value of any plant depends on bioactive phyto chemical components like phenolics, alkaloids, terpenes, flavonoids, tannins and saponins because these components produce specific physiological action in the human body [8]. Different compounds such as linalool, eugenol, methyl cinnamate, methylchavicol and citral are traded in international essential oil market, can be found in chemotypes of basil. These chemotypes are known by names on the basis of geographical origins such as French, Egyptian and European [2, 9]. Different essential oils have been reported to possess antibacterial activities against gram positive bacteria including *Staphylococcus aureus, Bacillus* species and gram negative bacteria namely *Escherichia coli, Salmonella*

enteritidis, *Shigella flexneri*, and pathogenic fungus specifically *Candida albicans* [10, 11].
Data regarding antibacterial activities of essential oil of different basil cultivars is very scanty in Pakistan. Present study was conducted to screen essential oils of Siam Queen basil, Holy basil and Italian basil for their antibacterial potential. Information generated could be exploited for further medicinal uses of basil.

2. Materials and Methods

2.1 Plant materials: Arial parts of Siam queen, holy basil and Italian basil including leaves and inflorescence were harvested from Herb Clonal Repository at Plant Genetic Resources Institute, National Agricultural Research Centre (NARC), Islamabad.

2.2 Essential oil extraction

Dried and ground aerial parts of the basil plants were subjected to hydro-distillation in a modified Clevenger type apparatus according to method outlined in the European Pharmacopeia (1996). Essential oil was collected from separating funnel and was stored at 4 °C prior to assay for antibacterial activity.

2.3 Bacterial Strains

Antibacterial activity was determined using disc diffusion method [12] with two gram positive strains (*Bacillus subtilis* and *Microbacterium lacticum*) and two gram negative strains (*Xanthomonas axonopodis*, and *Yersinia ruckeri*). Amoxicillin was used as positive control. Both antibiotic and essential oil were diluted according to following formula:

$$\frac{\text{Required concentration (ppm)} \times \text{required volume}}{\text{Stock concentration (ppm)}}$$

Bacterial spore suspension (10^5-10^6/ml) was prepared in autoclaved distilled water and added (10 mL) to nutrient agar media before solidification. After setting of media, sterile filter paper (Whatman No. 1) discs of 6mm in diameter were immersed in 10 µl of different concentrations (50 ppm, 100 ppm, 150 ppm and 200 ppm) of essential oil, dried and placed on the surface of the solidified nutrient agar media previously inoculated with microbial suspension. Filter paper discs having only DMSO were used as negative control. All the petri plates were then incubated at 37 °C for 24 hours, followed by measurement of zone inhibition expressed in mm. All the experiments were conducted in triplicate.

2.4 Statistical analysis

The results of antimicrobial activities were expressed as mean values of 3 replications. Analyses of variance were calculated using the statistical package Statistix 8.1 using least significant differences (LSD) at the 5% probability level. The statistical differences between means were calculated using Duncan's Multiple Range Test (DMRT).

3. Results

Analysis of variance showed that all dilutions of basil essential oils have significant antibacterial effect against all bacterial strains as shown in Table 1. Highly significant differences were recorded for essential oils/antibiotic, concentration, bacteria and oil x bacteria. Interactions for oil x concentration, concentration x bacteria and oil x concentration x bacteria were found statistically non-significant.

Mean values for zones of inhibition produced by three basil essential oils and antibiotic against four bacterial strains are presented in Table 2. Siam Queen basil essential oil produced bigger zones of inhibition against *B. subtilis* at 50 ppm, 100 ppm, 150 ppm and 200 ppm (2.6±0.23, 4.48±0.51, 4.1±0.21 and 4.33±0.23, respectively) than antibiotic (2.59±0.36, 3.16±0.39, 3.68±0.92 and 3.88±0.61, respectively). *Yersinia ruckeri* was also strongly inhibited by Siam Queen basil essential oil at 200 ppm concentration (4.33±0.47). It is clear from the results tha Siam Queen basil essential oil is more effective against gram positive bacteria (*B. subtilis*) as compared to other bacterial strains.
Holy basil essential oil also strongly inhibited the growth of *B. subtilis* at 200 ppm concentration and produced 4.03±0.04 mm zone of inhibition which is greater than the inhibition zone of

antibiotic (3.88±0.61 mm) at same concentration. *Xanthomonas axonopodis* growth was also strongly inhibited by Holy basil essential oil at 100 ppm and produced 4.25±1.80 mm zone which is approximately equal to the zone produced by antibiotic (4.38±0.08 mm).

Italian basil inhibited the growth of all bacterial strains moderately as compared to antibiotic. The largest zone of inhibition produced by Italian basil essential oil was against *Y. ruckeri* at 200 ppm (4.08±0.23) which varied greatly from the zone of inhibition produced by antibiotic at same concentration (7.73±0.57). Results showed that Italian basil is less effective against all bacterial strains as compared to holy basil and Siam queen.

Table 1: Analysis of variance for antibacterial activity of different concentrations of basil essential oils against gram +ve and gram –ve bacteria.

Source	Degree of freedom	Mean square (MS)	F
EO/Antibiotic	3	41.7141	198.70^{**}
Concentration	3	17.9378	85.44^{**}
Bacteria	3	10.8278	51.58^{**}
EO/Antibiotic × concentration	9	0.2134	1.02^{ns}
EO/Antibiotic × bacteria	9	7.5178	35.81^{**}
Concentration × bacteria	9	0.1842	0.88^{ns}
EO/Antibiotic × concentration × bacteria	27	0.3407	1.62^{ns}
Error	126	0.2099	
Total	191		

EO: Essential Oil

Table 2: Mean values for zones of inhibition produced by different basil essential oils and antibiotic against four bacterial strains.

Bacterial strains	EO/Antibiotic	Concentration (ppm)			
		50	100	150	200
M. lacticum	**Siam Queen**	2.16 ± 0.23^{yz}	$2.35\pm0.46^{u-z}$	$2.96\pm0.7^{n-x}$	$3.4\pm0.58^{i-r}$
	Holy Basil	$2.3\pm0.32^{w-z}$	$3.08\pm0.11^{l-u}$	$3.48\pm0.15^{i-p}$	$3.98\pm0.1^{f-j}$
	Italian basil	2.23 ± 0.7^{xyz}	$2.61\pm0.5^{s-y}$	$3.0\pm0.08^{m-w}$	$3.13\pm0.09^{l-t}$
	Antibiotic	4.37 ± 0.44^{fg}	5.29 ± 0.40^{d}	5.37 ± 0.43^{cd}	6.09 ± 0.07^{bc}
B. subtilis	**Siam Queen**	$2.6\pm0.23^{r-y}$	4.48 ± 0.51^{ef}	$4.1\pm0.21^{f-i}$	4.33 ± 0.23^{fg}
	Holy Basil	$2.31\pm0.2^{v-z}$	$3.06\pm0.09^{l-v}$	$3.41\pm0.37^{i-q}$	$4.03\pm0.04^{f-i}$
	Italian basil	$2.15\pm0.81^{y-z}$	$2.33\pm0.1^{v-z}$	$2.71\pm0.37^{q-y}$	$2.85\pm0.24^{o-y}$
	Antibiotic	$2.59\pm0.36^{s-y}$	$3.16\pm0.39^{k-t}$	$3.68\pm0.92^{g-n}$	$3.88\pm0.61^{f-k}$
X. axonopodis	**Siam Queen**	2.16 ± 0.08^{yz}	$2.85\pm0.14^{o-y}$	$3.08\pm0.06^{l-u}$	$3.55\pm0.35^{h-p}$
	Holy Basil	1.68 ± 0.5^{z}	$4.25\pm1.80^{f-h}$	$3.88\pm0.04^{f-k}$	$4.00\pm0^{f-j}$
	Italian basil	$2.6\pm0.43^{s-y}$	$3.58\pm0.42^{h-o}$	$3.71\pm0.57^{g-m}$	$4.08\pm0.23^{f-i}$
	Antibiotic	$4.01\pm0.03^{f-j}$	4.38 ± 0.08^{fg}	5.18 ± 0.06^{de}	5.4 ± 0.06^{cd}
Y. ruckeri	**Siam Queen**	$3.4\pm0.42^{i-p}$	$3.7\pm0.14^{g-n}$	$4.08\pm0.11^{f-i}$	4.33 ± 0.47^{fg}
	Holy Basil	$2.9\pm0.11^{o-x}$	$2.83\pm0.50^{p-v}$	$3.28\pm0.16^{j-s}$	$3.70\pm0.29^{g-n}$
	Italian basil	$2.5\pm0.4^{t-y}$	$3.50\pm0.30^{i-p}$	$3.75\pm0.36^{f-l}$	$4.05\pm0.47^{f-i}$
	Antibiotic	5.65 ± 0.47^{cd}	6.57 ± 0.15^{b}	7.41 ± 0.41^{a}	7.73 ± 0.57^{a}

Means followed by same letters are not statistically different at α=0.05

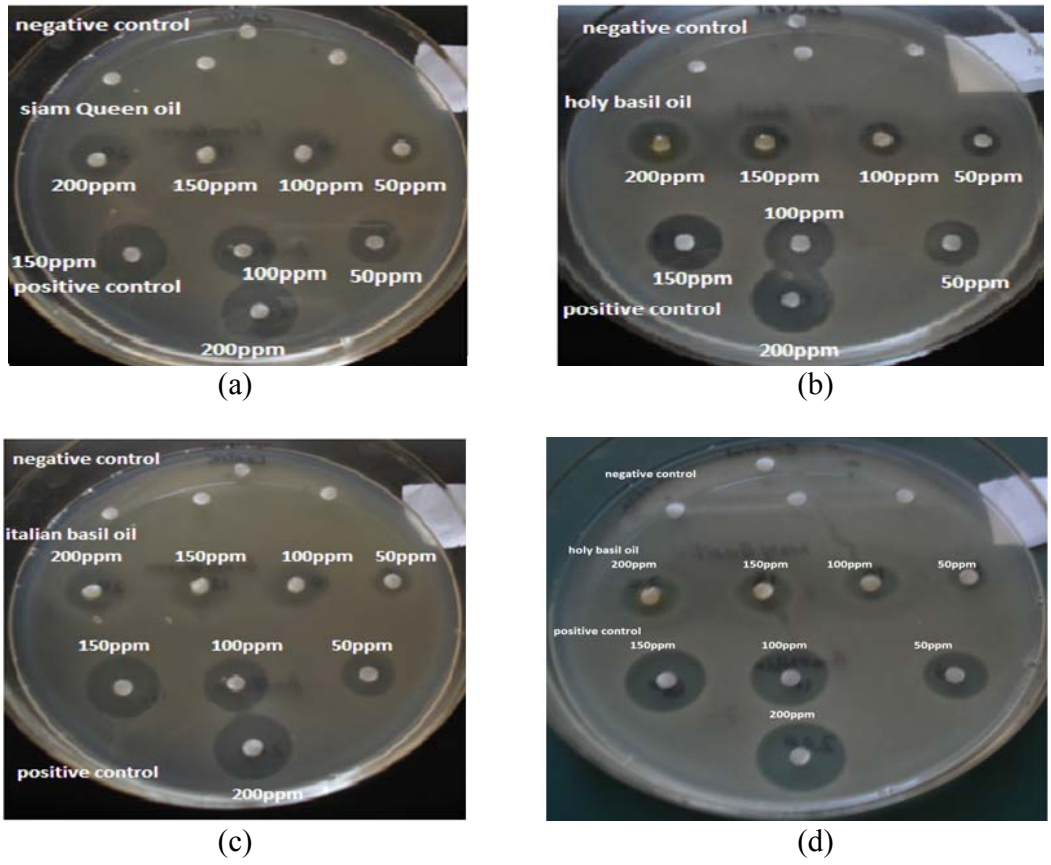

Fig 1: Antibacterial activity of (a): Siam queen against *Bacillus subtilis*. (b): holy basil against *Xanthomonas axonopodis*, (c): Italian basil against *Microbacterium lacticum*, (d): holy basil against *Yersinia ruckeri*.

4. Discussion

The presented data showed that basil essential oil is strongly effective against *B. subtilis* and moderately effective against *M. lacticum, X. axonopodis* and *Y. ruckeri* as compared to amoxicillin. Amoxicillin is recommended as the first choice-drug for bacterial sinusitis [13]. Amoxicillin is used as an antibiotic against gram positive and gram negative strains in number of infections such as pneumonia, skin infections and urinary tract infections. There is evidence in the literature that the essential oils of some Lamiaceae plants possess a moderate to good antibacterial activities [14, 15, 16]. However, in this study better effectiveness of essential oil inhibition was shown against gram positive bacteria i.e., *B. subtilis*. It may be due to absence of lipopolysaccharide layer in gram positive bacteria that may have acted as a barrier against any incoming biomolecule [17]. Previously, moderate antibacterial activity of *O. basilicum* essential oils have been reported [18]. Present results are in accordance with earlier reports in which Gram-positive bacterial strains were found to be more sensitive to basil essential oils as compared to Gram –ve bacteria [19, 20, 21]. This inhibition could be attributed to increased permeability of plasma membrane by essential oils, by inhibiting the microbial respiration, which causes the death of bacterial cell due to massive ion leakage [22]. Antimicrobial effects of basil essential oil could also be owed to the higher concentrations of linalool and eugenol [23, 24, 25].

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