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Comparative Analysis of *Ocimum basilicum* and *Ocimum sanctum*: Extraction Techniques and Urease and alpha-Amylase inhibition

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ABSTRACT
In the present work, two important medicinal plants of genus Ocimum, *O. basilicum* and *O.sanctum* have been compared in a number of phytochemical parameters. Effect of extraction techniques, solvent extraction, Soxhlet extraction and hydro-distillation, on the yield in different solvents have been investigated. Hydro-distillation gave a better yield of more volatile components than hexane fraction of the other two techniques. Both the plants showed good urease inhibitory activity. Hydro-distillate was stronger inhibitor than hexane or methanolic extracts of solvent or Soxhlet extractions. The extracts of *O. basilicum*showed a greater urease activity than extracts of *O. sanctum.*Alpha-amylase inhibitory activity of *O. basilicum* was also higher than that of *O. sanctum*. Notably, the synergistic effect of the extracts of the two plants was much higher than their individual efficacy against alpha-amylase. The activity decreased with decrease in concentration. Both the species had almost equal air pollution tolerance index (APTI) with *O. basilicum*(10.558) having slightly higher value than *O. sanctum* (9.202).Both plants contained alkaloids and phenolics. Both the plants also had almost same nutritional values.

Keywords: *Ocimumbasilicum, Ocimum sanctum*, extraction, urease, alpha-amylase, APTI

1. INTRODUCTION
The genus *Ocimum* belongs to the family Lamiaceae (Labiateae) and is well-known for its importance in perfume industry. There are conflicting reports about the number of species in the genus. According to some reports there are 50 to 60 species [1, 2]while some authors gave the number as large as 150.
Basil is the collective name for the plants of the genus[3]. *Ocimum basilicum* and *Ocimum sanctum* are the two most important species of the genus, which are most widely distributed and widely studied.

Ocimum basilicum L.,or Sweet Basil,is an evergreen herb and is believed to have originated in the warmer parts of the Indo-Malayan regions and is distributed in tropical and hotter parts of the Indo-Pakistan Subcontinent. Essential oils extracted from fresh leaves and flowers of the plant are used as additives in food, pharmaceuticals and cosmetics[4].It has got ethnomedicinal value of being used in the treatment of headaches, coughs, diarrhea, constipation, pain in the joints, warts, worms, asthma, enlarged spleen and kidney disorders. It is known to be stomachic, diuretic and anthelmintic as well as useful in disorders related to heart and brain. Majority of the aroma compounds from volatile extracts of *Ocimum basilicum* have antioxidant activity[4]. The seeds being mucilaginous treat chronic dysentery. They are also given with juices in cases of constipation and internal piles[5].

Ocimum sanctum, Holy Basil, is believed to have immense medicinal value by the local people[6]. In view of the wide range of its pharmacological applications like antioxidant[7], antidiabetic[8], antifungal[9], cardioprotection[10], immunostimulant[8], a number of scientists investigated the plant.

The present study was aimed to compare the two species in different aspects. The plant materials wereextracted in different solvents using different techniques and their quantities were compared. The extracts were subjected to preliminary phytochemical analysis, determination of nutritional values, and air pollution tolerance indices (APTI).Enzyme inhibition activity of plant extracts against α-amylase and urease was also studied.

2. MATERIALS AND METHODS

2.1 *The Plant Materials*
The leaves of *Ocimum basilicum* and *Ocimum sanctum* were collected from a local nursery of Lahore, Pakistan and identified by the authors. After removing dust and foreign particles, the fresh leaves were used for extraction.

2.2 *Chemicals*
All the chemicals used were of analytical grade and were purchased from E. Merck, Germany. α-amylase used was of fungal origin (*Aspergillus oryzae*) and purchased from UNICHEM, Turkey. Acarbose was purchased from Baeyer, Pakistan. Urease was of Jack Bean origin and was purchased from AVONCHEM. Throughout the study, double-distilled water and de-ionized water was used.UV-Visible Spectrophotometer UVD-3200Labomed, Inc. was used to measure absorbance.

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2.3 *Extraction by Different Techniques*

Solvent extraction, Soxhlet extraction and hydro-distillation techniques were used to extract essential oils and other chemical constituents from the leaves of *O. basilicum* and *O. sanctum*. Hexane, methanol, chloroform and diethyl ether were used as solvents.

Solvent Extraction Fresh leaves (500 g) were ground to obtain a paste which was soaked in 500 mL of hexane kept at room temperature for 14 days with occasional shaking and stirring with a glass rod. Then the extract was filtered and from the filtrate, hexane fraction was separated from the aqueous phase with separatory funnel. The hexane portion so obtained was dried over anhydrous Na_2SO_3 and solvent was recovered under reduced pressure using rotary evaporator. In this way hexane extract was obtained. The aqueous portion was extracted with chloroform to get chloroform extract. The plant residue was extracted with methanol for 14 days and methanolic extract was obtained using the same technique.

Soxhlet Extraction In Soxhlet extraction, a paste of the ground leaves (500 g) was extracted with 500 mL of hexane for 24 hours at 69°C.The plant residue and aqueous phase were extracted with methanol and chloroform to get their fractions.

Hydro-distillation In hydro-distillation the paste of the plant material (500 g) was soaked in distilled water and distillation was carried out giving 2 hr/50 g at 100°C. Essential oils were extracted from the distillate in diethyl ether with a separatory funnel.

2.4 *Phytochemical Analysis*

The extracts of both the plants were tested for the presence of alkaloids[11], carbohydrates[12], saponins, proteins and amino acids, fixed oils and fats[13], phenolic compounds and tannins[14]and gum and mucilages[15].

2.5 *Urease Inhibition*

Urease inhibition was carried out according to the reported method[16, 17]. The principle on which the method is based is that urease catalyzes the conversion of urea to ammonia which on reaction with salicylate, hypochlorite and nitroprusside forms a blue coloured compound called indophenol (λmax is 625 nm):

$$\underset{\text{Urea}}{H_2N\text{-}CO\text{-}NH_2} + H_2O \xrightarrow{\text{Urease}} NH_3 + CO_2$$

$$NH_3 + \text{Salicylate} \xrightarrow[\text{Nitroprusside}]{\text{NaOCl}} \text{Indophenol}$$

To determine the ability of an extract to inhibit urease, the extract (0.1 g) was dissolved in 25 mL of phosphate buffer (pH 7.0) and stored at 4°C. Then, different dilutions (5-100 µg/5mL) of this solution weremade to prepare test solutions of the extract. To a test solution taken in a testtube 15 µL of urea (0.08g/mL), 485 µL of phosphate buffer and 2.5 mL of Reagent 1(consisting of phosphate buffer 120 mmol/L, sodium salicylate 60 mmol/L, sodium nitroprusside 5 mmol/L, EDTA 1mmol/L, and urease 0.005g/L) was added, and after incubating the mixture at 37°C for 5 min, 2.5 mL of Reagent2(consisting of phosphate buffer 120 mmol/L, sodium hydroxide 400 mmol/L, and sodium hypochlorite 10 mmol/L) was added. The reactions mixture was incubated at 25°C for 10 min, and absorbance was noted at 625 nm on a UV-visible spectrophotometer against a blank. The blank contained 500µL of buffer and 2.5 mL of Reagent 1 and 2.5 mLof Reagent 2, which was added without prior incubation of the mixture. The standard contained all the reagents except the sample. The percent inhibition was determined using the formula:

$$\textbf{\% Urease Inhibition} = 100[(A_o - A_s)/A_o]$$

Here A_o is the absorbance of the standard, while A_s is the absorbance of the sample. Each experiment was repeated thrice and average was calculated.

2.6 *α-Amylase Inhibition*

Time-Dependent Inhibition: α-Amylase inhibitory activity of plant extracts by time-dependent method was analyzed by slightly modifying the reported method[18, 19]. Enzyme solution was prepared by dissolving 0.05 g of α-amylase in 100 mL distilled water. Potato starch (0.5%w/v) prepared in 20 mM phosphate buffer was used as substrate. DNS (dinitrosalicylic acid) reagent was prepared by mixing 96 mM 3,5-dinitrosalicylic acid and 12 g sodium potassium tartarate in 8 mL of 2M NaOH. To study the inhibitory effect of a sample, 120 µL of the sample (20 mg/mL in DMSO), 480 µL of distilled water and 1200 µL of the starch solution were mixed. The reaction was started by adding 600 µL of enzyme solution and the reaction was incubated at 25 °C for 3 min. After every one min, 200 µLwas removed from the reaction mixture and added into a tube containing 100 µL of DNS reagent. The tube was then kept in water bath at 85°C for 15 min. After cooling the mixture to room temperature, 900 µL of distilled water was added and mixed thoroughly. Absorbance of the reaction mixture was observed at 540 nm against a blank. The blank contained 600 µL of distilled water instead of the enzyme solution. For control, the plant extract was replaced by

120 µL of DMSO and hence represented maximum enzyme activity. For t=0 min, a separate experiment was performed. To determine the combined or synergistic inhibitory effect of the two plants, samples were prepared by mixing the extracts of two in equal concentrations.

From the maltose standard curve (0-0.4 % w/v), the amount of maltose generated in the samples was calculated. For calculating the percentage inhibition, following equations were used:

% Reaction = (Mean maltose in sample/Mean maltose in control) × 100

% Inhibition= 100 - % Reaction

Dose-Dependent Inhibition: α-Amylase inhibition in dose-dependent manner was carried out according to the reported method[20]. The starch solution (0.5% w/v) was taken as the substrate and was prepared by boiling 0.5 g of potato starch in 100 mL of distilled water for 15 min. The enzyme solution was prepared by dissolving 0.001 g of α-amylase in 100 mL of 20 mM phosphate buffer (pH 6.9). The test solutions were prepared in DMSO to give concentrations from 1.18 to 3.6 mg/mL. The DNS solution (20 mL 96 mM 3, 5-dinitrosalicylic acid, 12 g sodium potassium tartarate in 8 mL of 2 M sodium hydroxide and 12 mL deionized water) was used as the colour reagent of reaction. Three sets of experiments were conducted: test along with synergistic effect, blank and control. 1 mL test solution and 1 mL enzyme solution were mixed in a tube and incubated at 25°C for 30 min. Then 1 mL was taken out and 1 mL of starch solution was added and the tubes were incubated at 25°C for 3 min. Finally, 1 mL of the colour reagent was added and after covering the tubes, they were placed in water bath set at 85^{o}C for 15 min. The tubes were then cooled and the reaction mixture was diluted with 9 mL of distilled water. The absorbance value was recorded at 540 nm. In case of blank incubations, the colour reagent was added before the addition of starch solution. Rest of the method was same as for the test. For control incubations all procedure was again the same except that plant extract was replaced by 1 mL DMSO. Antidiabetic medicine acarbose (in concentrations of 0.0094, 0.0118, 0.0147, 0.0184, 0.023, 0.036, 0.056, 0.07, 0.11, 0.21, 0.42 µg/mL) was used as positive control. The percentage inhibition was calculated by the formula:

$$\%\text{Inhibition} = 100 \times [(A_c - A_s) / A_c]$$

Here A_c is the absorbance of control while A_s the absorbance of sample. The %Inhibition was plotted versus the sample concentration and a logarithmic regression curve was established to calculate the IC_{50} value for each sample which is the concentration of a sample required to inhibit the activity of α-amylase by 50%.

2.7 *Nutritional Evaluation*

Proximate nutritional evaluation was carried out according to the methods reported[21]. The moisture content was determined by drying the samples in an oven at 100°C until constant weight was attained. Ash was estimated by heating the samples in a muffle furnace at 600 °C until white ash was obtained. Proximate protein content was estimated by nitrogen determination using the Kjeldahl method[22]. Total lipid content was determined by Soxhlet extraction for 18 h at 160°C using hexane as the extracting solvent. Fibre content was evaluated by successive digestion of the defatted sample with 1.25% H_2SO_4 and 1.25% NaOH. Proximate carbohydrate estimation was made by the difference method[23]using the following equation:

Carbohydrates = 100- (ash + fat + protein + moisture + fiber)

Nitrogen free extract (NFE) was calculated according to the following expression:

NFE= 100 - (% Crude protein+ % Crude fat+ % Crude fiber+ % Ash)

Energy/Caloric value was calculated using the conversion factors[24]: 1 g of carbohydrates and protein each provides 4 Kcal while 1 g fat provides 9 Kcal of energy.

2.8 *Air Pollution Tolerance Indices (APTI)*

Air pollution tolerance indices of both plants were estimated according to the reported method[25]. In a detailed analysis, relative leaf water content[26], total chlorophyll content[27], leaf extract pH and ascorbic acid content[28]were determined and used in the formula to find out APTI of each plant.

3. STATISTICAL ANALYSIS

Statistical Analysis of the results was done by using SPSS (16.0) and GraphPad Prism 3.02 for Windows (GraphPad Software, USA). One-way analysis of variance (ANOVA) followed by Tukey's post test was used to assess the presence of significant differences ($P<0.05$) between the extracts.

4. RESULTS

Percent yield of extracts in various solvents of *O. basilicum* and *O. sanctum* obtained through different techniques is given in Table I. Results of the urease inhibitory potential of various extracts of the plants are displayed in Table II. Findings of the alpha-amylase inhibitory activities of the extracts of the plants determined through time- and dose-dependent methods are given in Tables III and IV. The results of nutritional values, preliminary phytochemical analyses, air pollution tolerance indices, and proximate nutritional values are shown in Tables V, VI and VII, respectively.

Table-1: Percent yield of the extracts in different solvents of *Ocimum basilicum* and *Ocimum sanctum*

S. No	Plant Species	Extraction Technique	% Yield in different solvents		
			Hexane	Methanol	Chloroform
1	*Ocimum basilicum*	Solvent Extraction	0.553	0.841	0.064
		Soxhlet Extraction	0.072	1.124	0.013
2	*Ocimum sanctum*	Solvent Extraction	0.353	1.263	0.024
		Soxhlet Extraction	0.104	0.742	0.013

The % Yield of essential oils extracted through hydro-distillate method was 0.70 for *O. basilicum* and 0.64 for *O. sanctum* which is higher than the hexane extracts of both the plants.

Table-2: Percent Urease Inhibition by Extracts of Ocimum species

S. No	Conc. (µg/5mL)	% Inhibition								
		Methanolic fraction			Hexane fraction			Hydro-distillate		
		O. b	*O. s*	Synergic Effect	*O. b*	*O.s*	Synergic Effect	*O. b*	*O. s*	Synergic Effect
1	100	97.18	73.23	70.42	98.65	98.64	93.24	98.46	95.40	90.77
2	80	95.77	71.83	69.01	97.280	97.30	91.90	97.92	92.31	89.23
3	60	94.36	70.42	67.60	95.95	95.95	90.54	96.93	87.70	89.22
4	40	81.69	69.01	66.19	94.60	94.60	89.19	96.90	87.70	88.77
5	20	78.87	63.38	64.78	93.24	91.90	78.38	96.46	89.23	87.69
6	10	73.23	61.97	63.38	91.89	90.54	77.03	96.40	87.70	87.65
7	05	71.83	60.56	61.97	90.54	89.20	74.32	95.40	87.79	86.23

Table-3: α-Amylase Percent Inhibition by *Ocimum basilicum* and *Ocimum sanctum* by Time-Dependent Method

S. No	Fractions	% Inhibition at t = 3 min		
		Ocimum basilicum	*Ocimum sanctum*	Synergistic Effect
1	Hexane	72.60	60.46	69.91
2	Methanol	96	87.5	66.66
3	Chloroform	37.37	56.52	22.22
4	Hydro-distillate	64.16	95.87	73.98

Table-4: α-Amylase Percent Inhibition by *Ocimum basilicum* and *Ocimum sanctum* by Dose-Dependent Method

S. No	Concn. (mg/mL)	% Inhibition					
		Hydro-distillate			Methanol Fraction		
		O. basilicum	*O. sanctum*	Synergic Effect	*O. basilicum*	*O. sanctum*	Synergic Effect
1	3.6	76.65	57.550	97.170	38.09	43.88	70.25
2	3.00	63.91	47.10	80.110	31.801	36.80	61.99
3	2.88	61.40	46.06	77.670	30.05	35.23	60.21
4	2.3	48.10	36.80	62.100	24.435	28.05	52.41
5	2.0	42.601	31.101	53.102	21.161	24.51	49.101
6	1.84	39.30	29.50	49.800	19.52	22.601	47.80
7	1.47	31.40	23.501	39.900	15.71	17.19	42.73
8	1.18	25.30	18.871	31.990	12.60	14.62	38.704

5. DISCUSSION

The aim of the present study was to carry out a comparative analysis of two important species of genus *Ocimum*, namely, *Ocimum basilicum* and *Ocimum sanctum* from various parameters such as the effect of various techniques on the quantity of the extracts in different solvents, preliminary phytochemical analysis, urease and alpha-amylase inhibitory effects, nutritional values, and air pollution tolerance indices.

Table-5: Preliminary Phytochemical Analysis of Extracts of *Ocimum basilicum* (O.b) and *Ocimum sanctum* (O.s)

S. No	Phytochemical Test	Methanol Fraction		Hexane Fraction		Hydro-distillate	
		O.b	O.s	O.b	O.s	O.b	O.s
1	Alkaloids, Hager's test	-ve	+ve	-ve	+ve	-ve	+ve
2	Saponins	-ve	-ve	-ve	-ve	-ve	-ve
3	Phenolic compounds Lead acetate test	+ve	+ve	+ve	+ve	+ve	+ve
	Alkaline reagent test	+ve	+ve	+ve	+ve	+ve	+ve
4	Gums and Mucilages	-ve	-ve	-ve	-ve	-ve	-ve
5	Sodium	0.01 ppm	0.01ppm	0.00	0.00	-	-
6	Potassium	0.01 ppm	0.01ppm	0.00	0.00	-	-

Table-6: Air Pollution Tolerance Indices of *Ocimum basilicum* and *Ocimum sanctum*

S. No	Parameters	*Ocimum basilicum*	*Ocimum sanctum*	Difference
1	Relative leaf water content %	86.57%	91.127%	*
2	Total Chlorophyll Content	26.3174 mg/g	26.2026 mg/g	**
3	Leaf Extract pH	7.63	7.8	**
4	Ascorbic Acid Content (AA)	0.056 mg/g	0.022 mg/g	NS
5	Air pollution tolerance index	10.558	9.202	*

NS: Non Significant. *: $p<0.05$; **: $p<0.01$

Table-7: Proximate Nutritional Values of *Ocimum basilicum* and *Ocimum sanctum*

S. No	Parameters	*Ocimum basilicum*	*Ocimum sanctum*	Difference
1	Moisture	95.5%	95.2%	**
2	Crude Fat	0.1%	0.09%	*
3	Ash	1.91%	1.9%	**
4	Crude Protein	1.2%	1.02%	NS
5	Crude Fiber	1.08%	0.2%	NS
6	Carbohydrates	0.21%	1.59%	NS
7	Total energy	0.2616 Kcal	0.4491 Kcal	NS
8	Nitrogen Free Extract	95.71%	96.79%	**

NS: Non Significant. *: $p<0.05$; **: $p<0.01$

5.1 *Percent Yield of Extracts*

The percent yield of different extracts obtained from *Ocimum basilicum* and *Ocimum sanctum*in different solventsextracted by three different techniques,i.e., solvent extraction, Soxhlet extraction and hydro-distillation is given in Table I. For *O. basilicum* methanol fraction obtained through Soxhlet extraction gave the highest percent yield (1.124%). The results are in agreement with the work reported[29] according to which the yield of the hydro-distilled essential oils is from 0.5%-0.8%. The results are also justified through another reported work[30]according to which the yield of the essential oils was 0.37% extracted from basil plants of Serbia and Montenegro. For *O. sanctum* maximum percent yield was of the methanol fraction obtained through Soxhlet extraction (0.742%). The results are in agreement with the reported work[31] according to which the yield of *Ocimum sanctum* essential oilswas 1.45% and *Ocimum basilicum* essential oil was 0.98%. The results are also in agreement with another work[32] which reports the yield of two *Ocimum basilicum* varieties to be 0.20% and 0.50%, respectively. The results showed that hexane fraction was obtained in greater amount for *Ocimum basilicum* when extracted through solvent extraction and methanol fraction was obtained more for *Ocimum sanctum* when extracted through the same technique. Soxhlet extraction gave more yield of methanol fraction in case of *Ocimum basilicum*. The yield of chloroform fraction was higher through both Soxhlet and solvent extraction for *Ocimumbasilicum*. In case of hydro-distillation, the yield was greater for *Ocimum basilicum*. The comparative study reveals that *Ocimum basilicum* has overall more essential oil content than *Ocimum sanctum* and solvent extraction gave better yield than Soxhlet extraction for both plants. However, for the extraction of more volatile substances from both the species hydro-distillation method proved to be more successful as it gave better yield than hexane extracts obtained through solvent and Soxhlet extraction.

5.2 *Urease Inhibition*

The results of urease inhibition are given in Table II. The hydro-distillate (98.46-95.40), hexane (98.65-90.54) and methanol (97.18-71.83) fractions of *Ocimum basilicum* showed higher urease inhibition (%) as compared to *Ocimum sanctum*. In synergistic studies, the anti-enzyme action was less than that of *O. basilicum* and almost equal to that of *O. sanctum*. The hydro-distillate of both the plants showed comparatively better inhibitory effect than the other fractions. However, when hydro-distillates of both the plants were used combined, they also showed less inhibitory activity. The hexane fractions that comprise more volatile components were comparatively weaker inhibitors of

urease. As expected, the urease inhibitory activity of the extracts of both the plants decreased with the decrease in concentration.

Both the species of *Ocimum* exhibited strong urease inhibitory activities, which are higher than many plants reported in other studies[16]. In view of these results it can safely be said that these medicinal plants can be used where required to inhibit activity of urease such as to control urease induced stomach ulcers[33].

5.3 *α-Amylase Inhibition*

Time-Dependent Method: The results of α-amylase inhibition through time-dependent method for both the plants are given in Table 3. The hexane (72.60%) and methanol (96%) fractions of *Ocimum basilicum* showed greater inhibition of α-amylase as compared to *Ocimum sanctum*, however, chloroform fraction (56.52%) and hydro-distillate (95.87%) of the latter were more effective than the former. The synergistic inhibitory effect of the two plants, in general, gave almost an average value in these experiments.

Dose-Dependent method: The results of dose-dependent method are shown in Table IV. Alpha-amylase inhibitory effect of all the extracts of both the plants decreased with the decrease in their concentration. Although a number of studies have been conducted on alpha-amylase inhibitory activities of *O. basilicum* and *O. sanctum*[34, 35, 36,11]but their synergistic effect has not been so far evaluated. In dose-dependent analysis, the synergistic effect was in general much higher as compared to their individual effectiveness. For instance, at concentration 3.6 mg/mL, efficacy (in percent) of hydro-distillate of *O. basilicum* and *O. sanctum* was 76.65 and 57.55 while their synergistic effect was 97.17, which was much higher than their average. In the same manner, the synergistic effect of the methanolic extract of the two plants (70.25) was much higher than their individual effects which were 38.09 and 43.88 respectively. These results are very encouraging and the plants can be further explored on these lines to develop appropriate herbal recipes to inhibit alpha-amylase activity in diabetic patients.

5.4 *Phytochemical Analysis*

The results of phytochemical analysis of *O. basilicum* and *O. sanctum* are given in Table V. For Thephenolic compounds and alkaloids were detected in hydro-distillate, hexane and methanolic fractions of both the plants. The results are in agreement with the work[37]which reports the presence of phenols in essential oils of *O. sanctum*

The sodium and potassium levels of methanolic and hexane fractions of *O. basilicum* and *O. sanctum* were same for both the plants. The results are in agreement with the reported work[38] which mentions the presence of trace elements in *Ocimum basilicum* and found Na and K in very small amounts. In some studies, presence of flavonoids has also been reported.

5.5 *Air Pollution Tolerance Indices*

The results of air pollution tolerance index (APTI) are given in Table VI. For *O.basilicum,* APTI was 10.558 whereas for *O.sanctum* it was 9.202. APTI of *O.basilicum* and *O.sanctum*were reported to be 12.28 and 12.16 respectively[39], which is higher than our results. However, the variation of results between the two species was the same i.e., *O.basilicum*was more tolerant to air pollution than *O.sanctum*. Since leaves are greater in number, more exposed to the environment and are the direct receptors of pollution, they are used in APTI studies. Relative leaf water content (RWC) tells about the healthy environment in which the plant is grown. The more the water content, the healthiershould be the plant tissues. Thus, *O.sanctum* having higher water content (91.127%) represented a healthier species. The chlorophyll content is related to the biomass development of a plant and has a relationship with the age of the plant. The plants having greater chlorophyll have greater biomass development. The chlorophyll content of *O.basilicum* is 26.3174 mg/g and that of *O.sanctum* is 26.2026 mg/g. It shows that biomass is slightly well-developed in *O.basilicum* than *O.sanctum*. The pH of the leaf extract shows the acidity or basicity of the pollutants. A plant material having acidic pH means it has higher pollutant levels. In the present study, the pH of the leave extract of*O.basilicum* was 7.63 and that of *O.sanctum* was 7.8 which were almost equal. In plants, ascorbic acid (vitamin C) content is related to SO_2 pollution. It is also a good radical scavenger and antioxidant. The data shows that *O.basilicum* was more resistant to sulfur dioxide pollution having 0.056mg/g ascorbic acid content as compared to 0.022mg/g value for *O.sanctum*. Overall, the parameters responsible for APTI were stronger in magnitude for *O.basilicum*.

5.6 *Nutritional Values*

The results of nutritional evaluation are given in Table VII. As the results show, the percentage of moisture (95.5%), ash (1.91%), crude fat (0.1%), crude protein (1.2%) and crude fiber (1.08%) was higher for *O.basilicum* as compared to *O. sanctum*. Percentage of carbohydrates (1.59%) and value of total energy (0.4491 Kcal) was higher for *O. sanctum*. Our findings are in agreement with those reported[37].

6. CONCLUSION

Ocimum basilicum and *Ocimum sanctum* are the two most important medicinal herbs of the family Lamiaceae. They possess notable urease and alpha-amylase inhibitory properties. They can find application in slowing urease catalyzed

decomposition of urea in the soil. They can be included in diet to cure stomach ulcer as well as to inhibit hydrolysis of starch into di-and mono-saccharidesthus giving relief to diabetic patients. Further studies on these lines may result in the discovery of a cheaper therapy for such ailments.

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