ANTIOXIDANT ACTIVITY AND TOTAL PHENOLICS CONTENT OF EXTRACTS FROM MURRAYA KOENIGII (CURRY LEAVES), LAURUS NOBILIS (BAY LEAVES), AND CAMELLIA SINENSIS (TEA)

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ABSTRACT

Antioxidant potential is related to polyphenolics content, the focus of present study was to estimate and evaluate total phenolics content (TPC) and antioxidant activity (AOA) of the cost-effective natural sources. For this purpose, leaves from three plant species including Murraya koenigii (curry leaves), Laurus nobilis (bay leaves), and Camellia sinensis (green and black tea leaves) were selected and analyzed. The AOA was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, Ferric reducing antioxidant power (FRAP) assay and iron(II) chelating activity (ICA). Methanol was used for the extraction of polyphenols from leaves. Results showed that the highest TPC (15300 mg GAE /100g) and the highest AOA (DPPH = 51 to 88%; FRAP = 24-85%; and ICA=19-74%) was found in the green tea leaves. The lowest TPC (170 mg GAE /100g) and the lowest AOA (DPPH = 20-64%; FRAP = 18-48%; and ICA=15-42%) were shown in curry leaves. Antioxidant activities were analyzed at different concentration ranges (50-250µg/100µl) and the result showed that AOA was concentration dependent. These leaves extract may be exploitable not only as a health supplementary, nutraceuticals values but also in the food preservation and packaging materials.

Key words: *TPC, DPPH, FRAP, AOA, chelating, green/ black tea, curry leave, bay leave.*

1. INTRODUCTION

Plant extracts are rich source of natural antioxidants and are being used as therapeutic agents [1], [2], nutraceuticals [3], and food preservatives [4]. One of the goals of natural product plant based research is to identify such phytochemicals whose potential novel action against deadly diseases can be unveiled. The naturally occurring antioxidants also used to replace synthetic ones. The side effects of synthetic antioxidant, for example, Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) are well known [5].

To stay healthy, human body needs well developed immune system with better resistant adaptability to fight against deadly bacteria and viruses. In this context, role played by balanced diet with important nutrients cannot be ignored. To evaluate antioxidant potential, different biological assays have been developed and are being used effectively. Similarly, various bioactive constituents present in the food, responsible for antioxidant activity, have also been identified from a number of plant species. Many fruits and vegetables have been screened for their antioxidant potential and total phenolics content. With the growing need of food nutraceuticals to fight against deadly diseases another challenge for researchers to meet with is to unveil cost-effective natural product antioxidant sources. Based on these facts, Murraya Koenigii, Laurus nobilis, and Camellia sinensis leaves were selected for the present study and their total phenolics content and antioxidant activity was evaluated.

During various metabolic activities, certain reactive oxygen species (ROS) produced in human body, are the root cause of different diseases, for instance, atherosclerosis, rheumatoid arthritis, cancer, etc. The damage caused by these free radical species is checked by various enzymes (catalase, superoxide dismutase, etc.) or compounds (phenolics, tocopherols, etc.) present in the body [6]. Antioxidant potential of phenolic compounds is a scientifically established reality now [7]. Based on these facts, edible plants are being screened for their naturally occurring antioxidant constituents. Pakistan's fertile land and better adapted canal system is the habitat of more or less 800 different plant species. It is reported that more than 90 nutritionally and medicinally important edible fruits in Pakistan are not consumed well. Edible fruits and vegetables are amongst the major sources of polyphenols. These polyphenolics attribute better quality (shelf life, taste, appearance) and therapeutic importance to food. In other words, these reduce oxidation reactions in food substances [8]. Hence, the objective of current study is to estimate polyphenolics content and evaluate antioxidant potential of some of these underutilized and economical plant species including Murraya koenigii (Curry leaf), Laurus nobilis (Bay Leaves), and Camellia sinensis (Tea).

M. koenigii is a well known leafy spice and used as a preservative in Asian-Indian cuisine. The smaller quantity of M. koenigii is sufficient to use due to its distinct aroma [9]. Besides reported dietary importance, medicinal potential of M. koenigii has also been established [10].

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Another important plant species used in the current study was Laurus nobilis (Bay Leaves). It is also used in cooking due to its peculiar flavor and fragrance in either fresh or dried form. Isolated compounds from L. nobilis have already been evaluated for their antioxidant potential [11]. It is cultivated in hilly areas of Pakistan.

Medicinally, L. nobilis is reported for the treatment of epilepsy, Parkinsonism, diabetes, etc. It is also a well known plant in Mediterranean cuisines. Preventive or therapeutic role of polyphenolics content of Camellia sinensis, (utilized as black, green or oolong tea) against several diseases is also reported [12]. Moreover, its antioxidant activity has also been evaluated earlier [13].

2. MATERIALS AND METHODS

2.1 Sample Preparation

Packets of (1 kg) Green, black tea (C. sinensis) and Bay leaves (L. nobilis) were purchased from the departmental store of Karachi, Pakistan. The curry leaves (M. koenigii) was picked from the garden at the University of Karachi in July 2014. After washing with water and air drying in open sieves, the leaves were stored at -5 °C. All the solvents and chemicals used were of analytical grades and supplied by either Merck or BDH.

2.2 Extraction of Polyphenols From Leaves

Previously reported procedure was followed for the extraction of polyphenols from the crude methanol extract [14].The sample size of all types of leaves were about 500g.

2.3 Determination of Total Phenolics

Total phenolics content was estimated in all four leaves extract following the procedure used by Jayaprakasha et al. [15] with slight modification as reported earlier [16]. Firstly 2 ml mixture of methanol and water (8:6) was prepared to dissolve the sample (12mg). After that 4 ml of (1:10 diluted) Folin-Cicolteu reagent was added in the solution with 4 ml of 10% sodium carbonate solution. The whole content (prepared mixture) was kept at 30^{o}C for 40 minutes then absorbance was measured at 567nm using Spectrophotometer (UV VIS Shimadzu). Gallic acid (GA) was used as a standard curve and result were presented in mg GAE/100g.

2.4 Antioxidant Activity

Three different protocols were followed for the determination of antioxidant activity of crude methanol extracts including DPPH, FRAP, and ICA.

2.4 Radical Scavenging Using DPPH Assay

The protocol developed earlier was used for the evaluation of DPPH scavenging potential with minor variations [17]. Different concentration samples (50-250 mg/100 µl) were prepared in methanol. The samples prepared were treated with 1.4 ml DPPH solution in methanol (0.2 mM) and 1.5 ml distilled water. After vigorous mixing the resultant mixture was placed in dark place for the interval of 30 minutes. Decreased absorbance of the samples was observed against blank at 515 nm using Spectrophotometer (UV-270 Shimadzu).

2.5 Ferric Reducing-Antioxidant Power (Frap)

This is based on the reduction power of all types of leaves extract from Fe^{+3} to Fe^{+2}. Previously reported protocol by Jayaprakashaet al.[15] for the estimation of ferric reducing power was followed.

Different concentration of samples were prepared in methanol (50-250 µg/ml) and 2.5 ml of both; phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferric cyanide were added. It was then followed by incubation of the samples prepared (for 20 minutes, maintained at a temperature of 50^{o}C). To this incubated mixture, 2.5 ml 10% trichloroacetic acid was added and the resulting mixture was centrifuged at 5000 rpm for 10 minutes. After adding 2.5 ml double distilled water and 0.5 ml 0.1 % $FeCl_3$ to the supernatant (2.5 ml), absorbance was measured at 595 nm. Reducing power was evaluated by increased absorbance of the samples in triplicate.

2.6 Chelating Effect

Chelating effect of the samples was evaluated using 2, 2-bipyridyl assay [18]. Methanol was used to prepare varying concentration of leaves samples (50-250 µg/ml). To 0.25 ml samples, 0.25 ml of 1mM $FeSO_4$ solution, 1 ml tris-HCl buffer (pH 7.4), 0.1% of 1ml 2,2µ-bipyridyl solution prepared in 0.2M HCl and 2.5 ml methanol were added. Fe^{2+} chelating activity of the samples was determined by measuring absorbance at 563 nm using standard Ethylene di –amine- tetra acetic Acid (Na_2EDTA) using Spectrophotometer (UV VIS Shimadzu)

2.7 Statistical Analysis

Statistical analysis was performed using samples in triplicates. Mean ± standard deviation was used to express data. Appling Pearson correlation test, relationship between antioxidant activity and total phenolics content was calculated. With the help of tukeys' test and analysis of variance (ANOVA) significant differences (at $p<0.05$) among the mean values were found.

3. RESULTS AND DISCUSSION

Studies of natural phenolics compounds have received more attention in the past few years because of its antioxidant properties. These antioxidant phenolics compounds reduce the toxic effect of reactive oxygen intermediates (ROI) generated by normal cellular metabolism in our body. Results of this study showed that

TPC was the highest (15300 mg GAE /100 g) in green tea followed by the black tea (12500 mgGAE /100 g), Bay leaves (550 mg GAE/100 g) and the lowest (170 mg/100 g) in curry leave (table 1).

Table 1: Total Phenols and antioxidant activity of C. sinensis (black), C. sinensis (green), M. koenigii and L. nobilis leaves. ($p< 0.05$) all values in mean (n=3).

Samples	**Total Phenols**	**Antioxidant Activity**		
µg/100µl	**mg/100g**	**%DPPH Scavenging**	**% Ferric Reducing**	**% Chelating Effect**
C. sinensis (black)				
50		63±1.55	55±1.09	51±2.09
100	12500	67±1.23	60±2.08	55±2.08
150		74±3.20	66±1.56	60±2.56
200		83±1.60	76±2.10	64±2.10
250		88±2.01	80±2.78	69±1.78
C. sinensis (green)				
50		75±2.30	61±1.89	56±2.09
100	15300	82±1.90	69±2.30	61±2.08
150		87±1.67	74±1.52	66±2.56
200		92±1.20	80±0.51	70±2.10
250		95±2.06	85±2.01	74±1.78
M. koenigii				
50		20±0.51	18±0.50	15±0.56
100	170	29±2.30	25±2.00	20±2.35
150		37±2.08	33±2.00	30±2.88
200		47±0.51	40±0.45	36±0.88
250		54±2.08	48±2.00	42±2.00
L. nobilis				
50		30±2.08	24±2.00	19±2.00
100	550	41±0.51	33±0.50	26±0.50
150		49±1.78	42±1.00	35±1.00
200		58±2.08	53±2.00	46±2.00
250		65±0.51	60±0.55	53±0.50

A previous study by Anesini et al. reported the total phenolics concentration 21.0 to 14.3 and 17.6 to 8.4 % of gallic acid equivalents (GAE) for green and black tea, respectively [19]. Other studies reported 27.1 to 25.7 and 22.3 to 17.5% GAE for green and black tea respectively [5]. Our findings are in similar range for green and black tea(15300 mg GAE /100 g and 12500 mg GAE /100 g) with the values reported. The minor variation in results may accounts for the natural parameters involved, including genetic composition and cultivar or variety difference. Other responsible factors include release of polyphenol oxidize during cutting or rolling of tea shoots. This enzyme interacts with polyphenolics content and reduces its concentration [5]. Besides above discussed factors, different quantification and extraction procedures adapted, can also contribute in the difference of polyphenolics content [20].

With some exceptions, previous studies reported that total phenolics content extracted better in methanol compared to ethanol and water. This is because methanol inhibits polyphenol oxidase which is responsible for the oxidation of phenolics content and its reduced amount. Another reason for choosing methanol over ethanol and water is its ability to evaporate easily[21].

The results of this study showed that C. sinesis (green) exhibited the highest antioxidant potential whereas M. koenigji showed the lowest (C. sinesis (green) > (black) >L. nobilis>M. koenigji). The numerical values obtained for C. sinesis (green and black), L. nobilis, and M. koenigji were < 20mg/100 µl, 20-30 µg/100 µl, 150 mg/100 µl (Fig. 1 and Table 1) respectively.

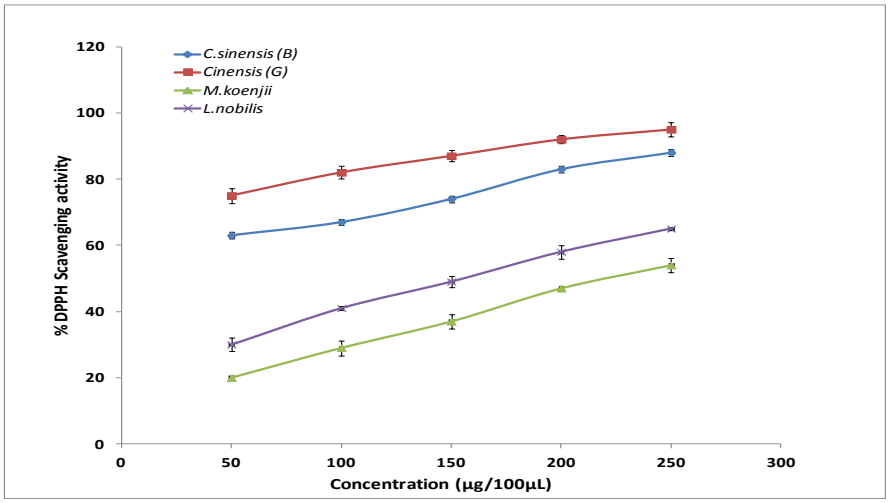

Figure 1: % DPPH scavenging activity of the crude Polyphenolic extracts derived from C. sinensis (black), C. sinensis (green), M. koenigii and L. nobilis leaves.

The antioxidant potential of green tea has been estimated to be higher as compared to black tea [19].

Among the all types of herbal leaves tested, the highest DPPH activity was shown by C. sinensis –green (green tea), C. sinensis-black (black tea) >L. nobilis>M. koenjii. The IC_{50} values for them < 20 µg/100µL, 20-30 µg/100µL, ~ 150 µg/100µL and 200-250 µg/100µL respectively. The IC50 value reported previously for L. nobilis (90 µg/100µL), was found to be much lower [22] compared to this study. Similarly antioxidant activity for M. koenigii leaf was found to be 116 µg/100µL [23] while some other studies on M. koenigii estimated the value of IC_{50} to be 0.006 mg/100µL, [24] which was lower compare to our experimental values (table 1). Like DPPH, % FRAP and % chelating effect followed the same pattern and same order of effect among all four types of herbal leaves. A strong correlation was observed between the concentration and antioxidant activity

As mentioned previously the most probable reasons for these variations in the values may be due to the fact that phenolic compounds may be water-soluble, lipid soluble, insoluble, or bound to cell walls. Therefore the efficiency in extraction is very important factor in quantitative analysis of AOA of food samples besides the natural occurring causes of the variation

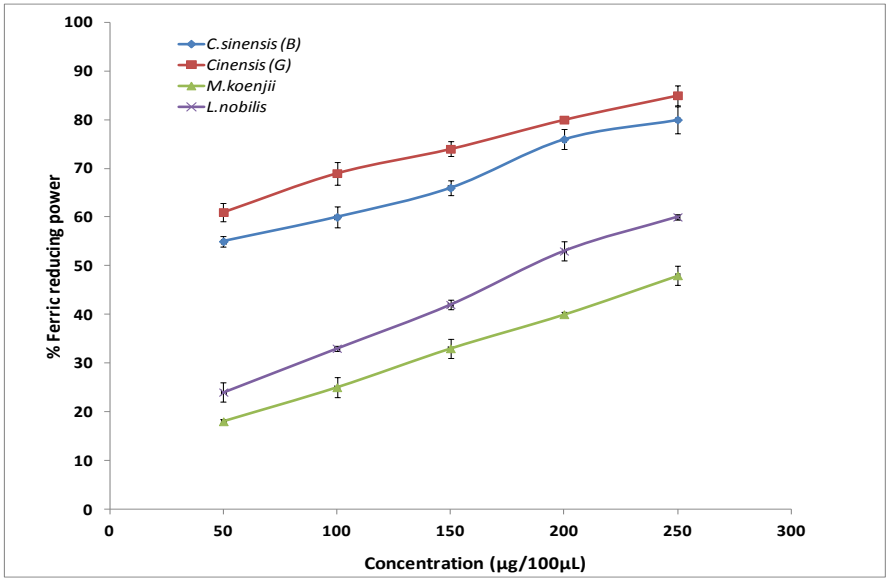

Figure 2: % Ferric reducing power of the crude Polyphenolic extracts derived from C. sinensis(black), C. sinensis(green), M. koenigiiand L. nobilis leaves,$p<0.05$ and all values in triplicate(n=3)

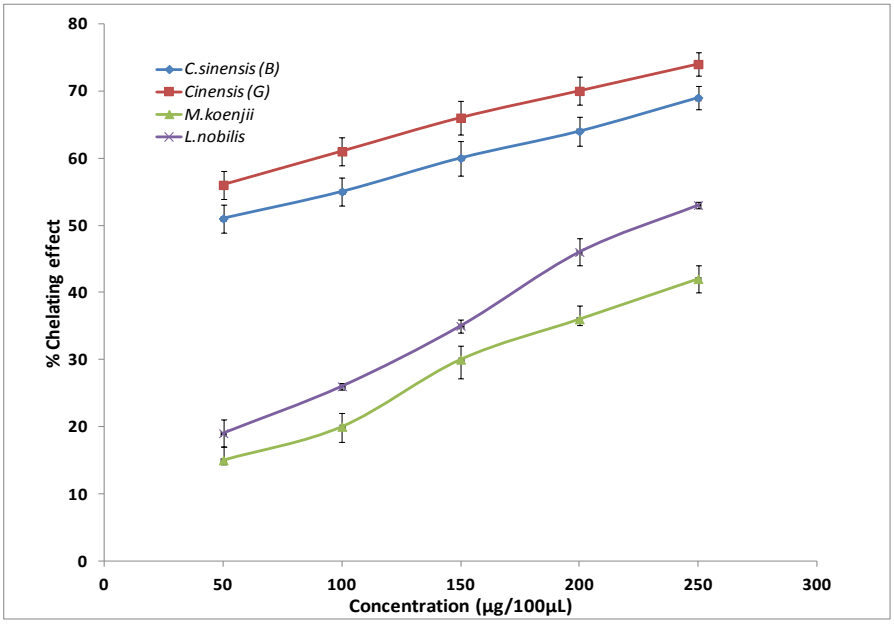

Figure 3: % Chelating power of the crude polyphenolic extracts derived from C. sinensis(black), C. sinensis(green), M. koenigiiand L. nobilis leaves,all values in triplicate n=3($p<0.05$)

4. CONCLUSION

Among the three tested species; M. koenigii, L. nobilis, and C. sinensis (green and black leaves), the .total phenolics .content was found highest for C. sinensis-green (15300 mg GAE /100 g). All three methods used for determining the AOA showed increase in AOA with increase in the concentration of methanol extract. Our result indicated great scavenging activity of the leaves tested against reactive oxygen species (ROS).These plants may be good source of natural antioxidants. The results obtained from this study can be utilized in food, pharmaceutical and packaging industries. Food scientist and technologist are still working on exploration of more stable and economical resources of polyphenols.

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6. CONFLICT OF INTEREST

No conflicts of interest were found during the preparation of this manuscript.

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