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Determination of total phenolic, flavonoid content and free radical scavenging activities of common herbs and spices.

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Abstract
Antioxidants present in herbs and spices could be an effective tool to prevent the non-communicable diseases like cancer, diabetes and myocardial infarction as they have the capacity to stabilize the free radicals which are one of the causative factors of these diseases. This study aims to quantify the total phenolic and flavonoid content of commonly used herbs & spices and determination of their free radical scavenging activities expressed as inhibitory concentration (IC_{50}). The results of this study depict that the tested herbs & spices have considerable amount of phenols and flavonoids and a high scavenging power of free radicals.

Keywords: Herbs, Spices, Hot water extracts, Phenols, Flavonoids, free radicals.

1. Introduction
Traditionally in all the cultures of the world herbs and spices are used as flavoring agents in cookery as well as they are medicinally used to treat various ailments [1]. Spice could be a dried bark, root or vegetable substance of a plant while herb is termed as the part of leafy green plant such as fruit, berries or any herbaceous plant [2]. Herbs and spices contain phytochemicals commonly known as antioxidants such as polyphenols, phenolic acids, tannins, flavonols, isoflavones and curcuminoids [3]. The high proportion of these antioxidants is the reason of various health benefits imparted by the herbs and spices [4]. The free radicals produced as a result of various metabolic processes taking place in the body are one of the important causes of diseases like cancer, diabetes, dementia and myocardial infarction as they interact with cellular DNA and cause its mutation [5]. The antioxidants present in herbs and spices are capable of neutralizing these free radicals by donating required number of electrons to stabilize them. Once the free radicals are stabilized after the acceptance of electrons, they become non-reactive to cellular DNA [6]. Herbs like coriander, bunching onion, curry leaves and holy basil & spices like ginger, onion, clove and lemongrass in three different combinations such as mixed herbs, mixed spices and mixed spices & herbs are used in this study to draw their antioxidant profile.

2. Materials and Methods
2.1. Chemicals and Standards
Gallic acid, Quercetin, Folin-Ciocalteu reagent, Sodium carbonate, 2,2- Diphenyl-1-picrylhydrazyl(DPPH), Sodium nitrate, Aluminium chloride, Sodium hydroxide, Methanol and distilled water.

2.2. Instruments
Grinder, Sonicator, Magnetic stirrer equipped with heater, refrigerator, Schott UVLine 9400 ultraviolet Spectrophotometer.

2.3. Collection and Preparation of Plant Material
All the herbs and spices used for this study were purchased from local market, washed with distilled water, air dried and then freeze dried. The freeze dried herbs and spices were then ground to fine powder which is then mixed homogeneously to get three formulations of herbs & spices. The first formulation F1 comprised of mixed herbs that is 25% each of powdered

curry leaves, holy basil, coriander and bunching onion, second formulation F2 comprised of mixed spices that is 25% each of powdered onion, ginger, clove and lemongrass while the third formulation F3 was the mixture of above two consisting of 12.5% powder of each of the 8 herbs and spices used in the study.

2.4. Extraction
All the three formulations of herbs and spices were homogenized separately in distilled water by using sonicator and then extracted by using magnetic stirrer equipped with heater set at 95 ºC for 4 hours. The extracts obtained were then filtered with Whatman filter paper No.42 and then stored in refrigerator set at 2-8 ºC for further use [7].

2.5. Preparation of Standard Solution
1 g of Gallic acid was dissolved in 100 ml of methanol to get 1% solution of Gallic acid (10 mg/ml) termed as standard 1 solution. Similarly 1g of Quercetin was dissolved in 100 ml of methanol separately to get 1% solution of Quercetin (10 mg/ml) termed as standard 2 solution.

2.6. Determination of Total Phenolic Content (TPC)
The total phenolic content of all the three formulations of herbs and spices was determined by using Folin- Ciocalteu method [8]. A standard gallic acid curve was constructed by preparing the dilutions of (0.1, 0.5, 1.0, 2.5 and 5 mg/ml) in methanol from standard 1 solution of gallic acid. 100 µl of each of these dilutions were mixed with 500 µl of water and then with 100 µl of Folin-Ciocalteu reagent and allowed to stand for 6 minutes. Then 1ml of 7% sodium carbonate and 500 µl of distilled water was added to the reaction mixture. The absorbance was recorded after 90 minutes at 760 nm spectrometrically. The same procedure was repeated with the pure hot water extracts of all the three formulations. The total phenolic content of the herbs and spices was calculated as gallic acid equivalents (mgGAE/g). All the experiments were performed in triplicate. Table.1 shows the mean absorbance of various concentrations of gallic acid and Fig.1 shows the standard gallic acid curve and regression equation used to calculate total phenolic content of the extracts.

Table 1: Absorbance of Standard (Gallic Acid)

Concentration (mg/ml)	Absorbance (Mean) λmax= 760nm
0.1	0.091
0.5	0.224
1.0	0.454
2.5	0.987
5.0	1.105

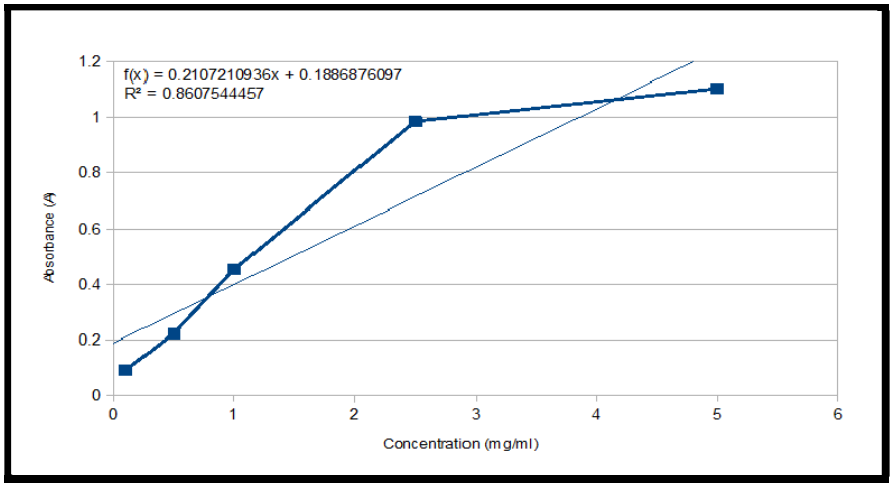

Fig 1: Standard curve of Gallic acid (TPC).

2.7. Determination of Total Flavonoid Content (TFC)
Aluminium chloride complex forming assay was used to determine the total flavonoid content of the extracts [9]. Quercetin was used as standard and flavonoid content was determined as quercetin equivalent. A calibration curve for quercetin was drawn for this purpose. From the standard 2 quercetin solution the dilutions of (0.1, 0.5, 1.0, 2.5 and 5mg/ml) concentrations were prepared in methanol. 100 µl of each of the quercetin dilution was mixed with 500 µl of distilled water and then with 100 µl of 5% Sodium nitrate and allowed to stand for 6 minutes. Then 150 µl of 10% Aluminium chloride solution was added and allowed to stand for 5 minutes after which 200 µl solution of 1M Sodium hydroxide was added sequentially. The absorbance of this reaction mixture was recorded at 510 nm on UV spectrophotometer. The same procedure was repeated with the pure hot water extracts of herbs and spices and total flavonoid content was calculated as quercetin equivalents (mgQE/g). All the procedures were performed in triplicate. Table. 2

Table 2: Absorbance of Standard Compound (Quercetin)

Concentration (mg/ml)	Absorbance(Mean) λmax=510 nm
0.1	0.285
0.5	0.345
1.0	0.478
2.5	0.799
5.0	1.399

shows the mean absorbance of various concentrations of quercetin while figure.2 shows the standard quercetin curve and regression equation used for the calculation of total flavonoid content of the extracts.

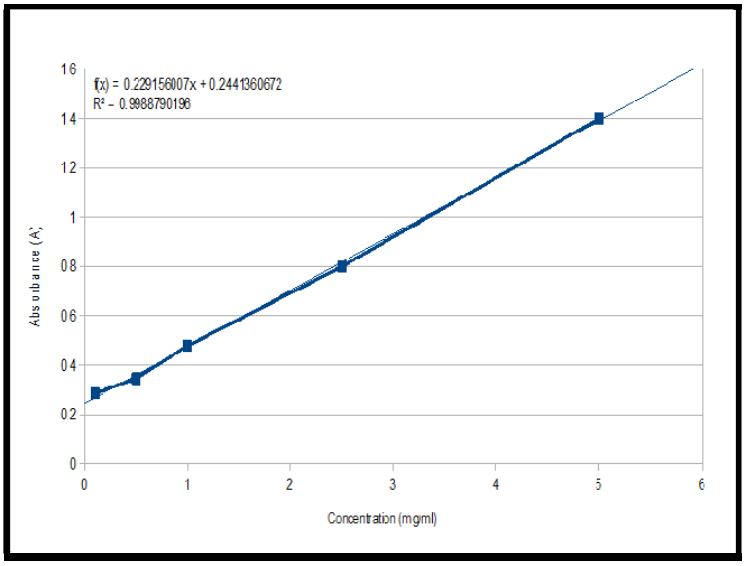

Fig 2: Standard Curve of Quercetin (TFC)

2.8. Scavenging activity (DPPH) assay

The free radical scavenging activities of the extracts were determined by using 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method [10]. DPPH in oxidized form gives a deep violet color in methanol. An antioxidant compound donates the electron to DPPH thus causing its reduction and in reduced from its color changes from deep violet to yellow. A fresh 0.002% solution of DPPH was prepared in methanol and its absorbance was recorded at 515 nm. 50 µl of pure extracts was mixed with 3 ml solution of DPPH and allowed to stand in darkness for 15 minutes. The absorbance was again recorded at 515 nm. The percentage inhibition of DPPH by extracts was calculated by using following formula

$$\% \text{ Inhibition} = \frac{A - B}{A} \times 100$$

Where A is the absorbance of pure DPPH in oxidized form while B is the absorbance of sample taken after 15 minutes of reaction with DPPH. A calibration curve of percentage inhibition of gallic acid was drawn for concentrations (20, 40, 60, 80 and 100 µg/ml) to determine the IC_{50} values of extracts that is the concentration at which 50% of DPPH solution is scavenged. All the experiments were performed in triplicate. Figure. 3 shows the standard curve of gallic acid and regression equation used to calculate the IC_{50} values of the extracts.

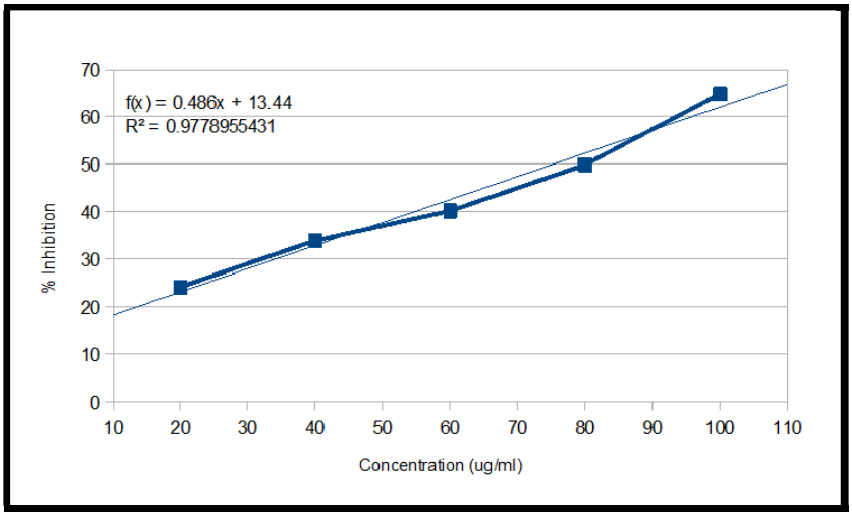

Fig 3: Standard Curve of Gallic acid (DPPH)

3. Results and Discussion

As mentioned above three formulations of herbs and spices were tested for the determination of total phenols, total flavonoids and their free radical scavenging activities. The total phenolic content of all the three formulations of herbs and spices varied from 0.54 ± 0.2 to 1.83 ± 0.3 mg/g of freeze dried plant material. The formulation F2 comprising of mixed spices showed the maximum phenolic content of 1.83

± 0.3 mg/g .The total flavonoid content of the three formulations of herbs and spices tested varied from 0.24 ± 0.01 to 0.40 ± 0.1 mg/g of freeze dried plant material. Maximum flavonoids were found in formulation consisting of mixed herbs (F1) showing the flavonoid content of 0.40 ± 0.1 mg/g. Table. 3 describes the TPC and TFC of all the three formulations of herbs and spices while figure.4 shows their graphical presentation.

Table 3: Total Phenolic and Flavonoid content of Hot Water Extracts of Herbs and Spices.

Formulations	Total Phenolic Content (TPC) mg/g	Total Flavonoid Content (TFC) mg/g
Mixed Herbs(F1)	0.54 ± 0.2	0.40 ± 0.1
Mixed Spices(F2)	1.83 ± 0.3	0.24 ± 0.01
Herbs + Spices(F3)	0.92 ± 0.1	0.29 ± 0.1

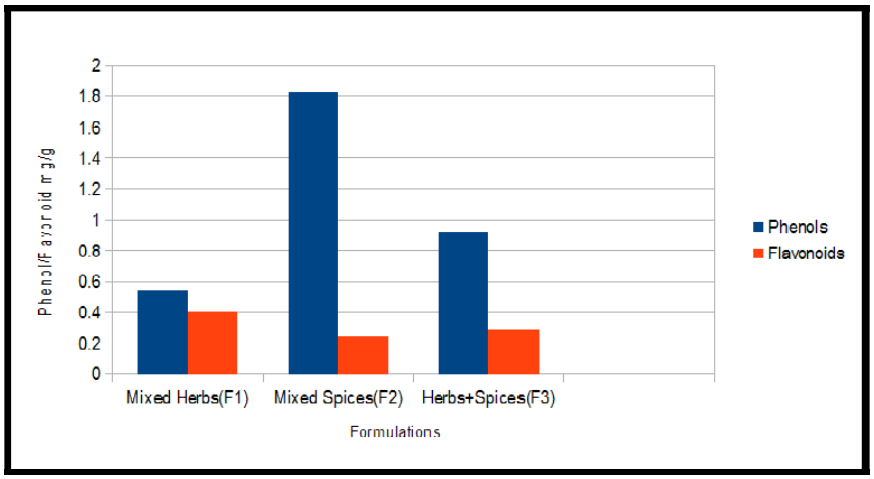

Fig 4: TPC & TFC of Herbs and Spices.

DPPH free radical scavenging method was used to determine the concentrations of extracts at which they scavenge the 50% of the DPPH solution termed as IC_{50} values. Gallic acid was used as standard for this purpose. The lower the IC_{50} value of an antioxidant the higher would be its free radical scavenging power. The mean IC_{50} of gallic acid was 75.6 ± 0.3. The IC_{50} values of the herbs and spices varied from 87.2 ± 0.2 to 87.6 ± 0.1. The formulation F1 comprising of mixed herbs showed highest scavenging activity for DPPH with mean IC_{50} value of 87.2 ± 0.2. Table 4 describes the IC_{50} values of the samples tested with DPPH while figure.5 describes the graphical comparison of the IC_{50} values of gallic acid with those of extracts.

Table 4: DPPH (IC_{50}) values of Gallic acid & Hot water extracts of Herbs & Spices.

Samples	IC_{50} (µg/ml)
Gallic acid	75.6 ± 0.3
Mix Herbs(F1)	87.2 ± 0.2
Mix Spices(F2)	87.6 ± 0.1
Herbs + Spices(F3)	87.5 ± 0.2

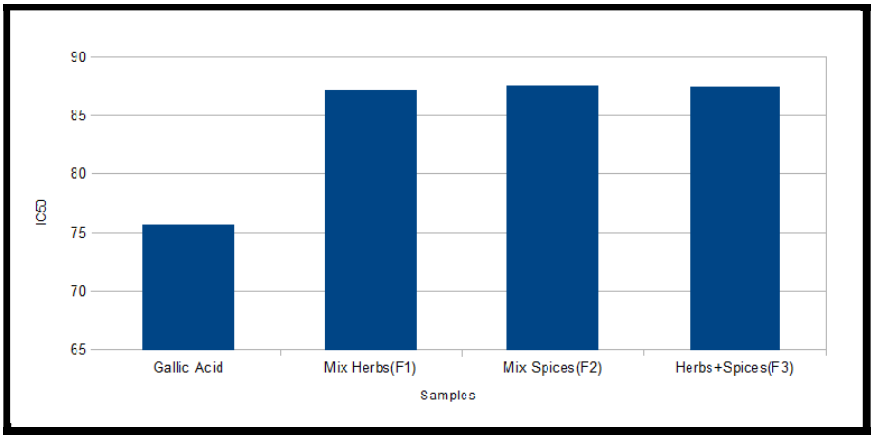

Fig 5: IC_{50} values of Gallic acid & Extracts.

The experimental findings show that hot water extracts of herbs and spices contain considerable amounts of phenols and flavonoids due to which their free radical scavenging power is comparable with gallic acid. The findings of this study specify the importance of herbs & spices as a readily available source of antioxidants in order to prevent the occurrence of non- communicable diseases like cancer, diabetes, dementia and myocardial infarction for which free radicals are considered one of the major contributing factors.

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