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Determination of antioxidants and phenolic contents in curry and Tulsi leaves

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Abstract
Leaves, an unavoidable component of plants are not a part of only accessories. They are loaded in pharma and nutraceutical properties and have even cosmetic uses. But from the ancient time herbal leaves like curry and Tulsi leaves it is very common to pick up leaves from dishes and put aside before even knowing about its health benefits. They are completely loaded with phenols, flavonoids and different antioxidant and other properties. The essential advantages found in leaves is their therapeutic uses in different ailments are their safety besides being inexpensive, valuable and their easy availability. The requirement of the study was to know the important compounds present in leaves useful to human health. Proximate and chemical analysis of Murraya koenigii and Ocimum basilicum leaves was investigated. The result shows that moisture content (65%-67% and 85%-87%), ash content (9.5%-11% and 14.5%-15.5%), ascorbic acid (4mg-4.5mg and 3.5mg-4.5mg), TPC (total phenolic content) (1.266-1.267 and 1.265-1.269) and DPPH scavenging activity (50%-51.8% and 52%-53%).

Keywords: Leaves, flavonoids, antioxidants, phenols, medicinal properties

Introduction

Leaves are important because of their natural components and have been used in various areas such as condiments herbal foods, medicines, non-alcoholic beverages etc. they can also be used in skin diseases, cold, cough, as anti-inflammatory, anti-microbial and antiseptic. (Hakkim *et al*., 2007 and Suppakul *et al*., 2003) [1, 2] anti-viral. (Umadevi, 2001). Phytochemicals are extensively found at diverse levels in many medicinal plants. The plants used in traditional medicine are useful in treating numerous ailments caused by oxidative stress, bacterial or viral infections. The huge number of edible plants used as foods and medicines by the Indian population devlops opportunities for the finding of novel phytochemical active compounds (Bhandari *et al*., 2012). There are numerous Indian medicinal plants with a mixture of pharmacological activities because they are present with a diversified class of phytochemicals (Garg *et al*., 2010). Leaves extract showed the protective nature in diabetes by diminishing pancreatic beta cell damage and oxidative stress (Arunselvan and Subramanian, 2007). Research have shown that many herbal leaves contain many anti-microbial, anti-allergic and many anti-oxidant properties. The importance of phenolic acid and flavonoids act as natural antioxidants has been concern due to their pharmacological and nutraceutical behavior. (Chen JH *et al*., 1997).

Material and methods

Procurement of leaves

The leaves were extracted from local market of prem nagar, dehradun and then they were washed properly to remove all the dust and unwanted particles. Then the leaves were set to dry in tray dryer at 70 degree Celsius for 3-4 hours and the leaves were grind to form powder and to determine all the proximate analysis.

Moisture content

Moisture content was resolute by the standard method of ranganna, 1986. The moisture content is defined as the amount of water present in fruits, vegetables and other edible raw products. For determining the moisture content in the sample, dry empty petri dish is weigh and then 2gm sample is placed in it and it is kept in hot air oven at 110 degree Celsius for 2-3 hours. After the given time the petri dish are kept in the desiccators to cool down and the weight is taken using weighing machine. Calculation is done by the formula:

$$\text{Moisture content (\%)} = \frac{W_3 - W_2}{W} \times 100$$

Where, w = weight of sample w_2= weight of petridish + sample w_3 = weight of petridish after drying.

Ash content

Ash content was firm by the standard method of rangnna, 1986. The ash content is defined as the amount of fiber, vitamins, minerals present in fruits and vegetables. For determining the ash content, take weight of empty crucible and put 2gm of sample in it. Put the crucible on heating mantle and churn it till white smoke stop coming. Put the churned crucible in the muffle furnance at 550 degree Celsius for 4-5 hours. After the given time the crucible is placed in desiccator for cooling the crucible and weigh the crucible using weighing machine.
Ash content is calculated by

$$\text{Ash content (\%)} = \frac{W_3 - W_2}{W} \times 100$$

Where, w = weight of sample W_2 = weight of empty crucible W_3 = weight of crucible after ashing.
Ascorbic acid (vitamin c)
Reagents 2, 6- Dichlorophenol indophenols solution: 52mg

of the sodium salt of dye and 42mg of sodium bicarbonate is make upto 500ml using distilled water.
Meta phosphoric acid (6%): 6% Meta phosphoric acids were prepared by dissolving 60gm in distilled water and make the volume 1000ml.Standard ascorbic solution: Standard solution was prepared by dissolving 10m L-Ascorbic acid in 6% Meta phosphoric acid solution and then make up the volume up to 1000ml.

Procedure

2ml of the extract was mixed with 6% metaphosphoric acid. It was then transfer into 20ml standard vitamin C solution in conical flask. The solution was then titrated against the dye solution till it shows the presence of a light pink colour. Note down the volume of the dye used, (x ml). Then again titrate the 20ml of sample solution against dye solution and record the volume of dye used, say y ml. Amount of ascorbic acid in 100ml of undiluted extract was determined by using the formula.
Ascorbic acid (Vit. C) (mg/100ml) = (y/x) × 10mg

Total Phenolic contents

(TPC) Total phenolic content was calculated by the method given by Makkar, H.P.S., *et.al,* 2007.
Reagents
Standard Gallic acid solution: Standard Gallic solution was made by taking 50mg Gallic acid and volume make up to 100 ml with distilled water.
Folin-Ciocalteu solution: 10% Folin-Ciocalteu solution was made by mixing 1ml folin-ciocalteu solution in 9ml distilled water.
Sodium carbonate solution (Na_2CO_3): 20% Na_2CO_3 solution was prepared by taking 20gm sodium carbonate and volume make up to 100ml with distilled water.
Procedure
In a ml juice sample 0.5ml folin-ciocalteu was added and then 2.5 ml Na_2CO_3(20%) was dissolve in test tubes after vortexing the mixture by using vortex shaker and the test tubes are kept in dark for 40-45 minutes and absorbance was taken at 725nm with UV-VIS spectrophotometer.
Preparation of standard curve
A standard curve of phenolic content was plotted in the range of 50-500 mg GAE/L by taking 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 mg/ml in test tube and volume make up to 1ml with distilled water after that 0.5 ml of folin-ciocalteu and 2.5 ml (20%) sodium carbonate was added in each test tube and kept in dark place for 40-45 minutes and absorbance was taken at 725nm.

DPPH

The (2,2- diphenyl-1-picryl hydrazyl) DPPH assay, is a organic chemical compound, is the most ordinary method of antioxidant activity assessment and which determine the capability of compounds to transfer labile H- atoms to radicals,. (Brand-Williams *et al*., 1995) [8]. It can be easily measured by spectrophotometer under 515-528 nm wavelength
DPPH Reagent: It was prepared by taking 0.004% DPPH by measuring 10 mg (0.1gm) of DPPH reagent in 250 ml of methanol (90%). Its absorbance was taken in UV- Visible spectrophotometer at 517nm. Absorbance should be approx 0.98.
Procedure
Sample was prepared by measuring 0.57gm of extract in 11.5 methanol (80%). From the stock solution aliquots were withdrawn and were prepared by mixing 10, 20, 30, 40, 50, 60, 70, 80 ul and aliquots from the stock solution with 990, 980, 970, 960, 950, 940, 930, 920 ul of methanol in each test tubes correspondingly. After that add 4ml of 0.004% DPPH to each sample in the test tubes and then in normal room temperature in dark incubate the sample for 30 mins. After 30mins incubation, the absorbance was measured at 517 nm using a UV Visible spectrophotometer.
Inhibition activity of free radical DPPH percentage (IA %) was calculated using the following correlation:

$$IA = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}$$

Result

After all the test performed the following results were analysed and they are presented in graphical representation as following,
The graphical representation of TPC (total phenolic content):

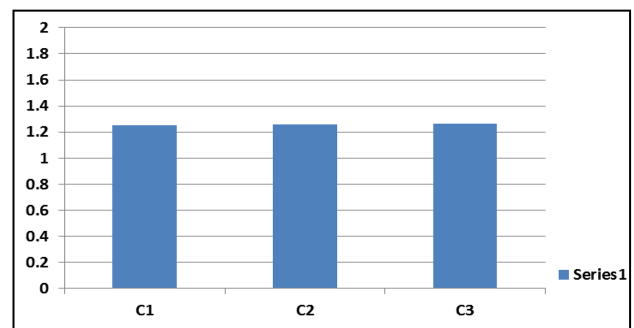

Fig 1: Murraya koenigii

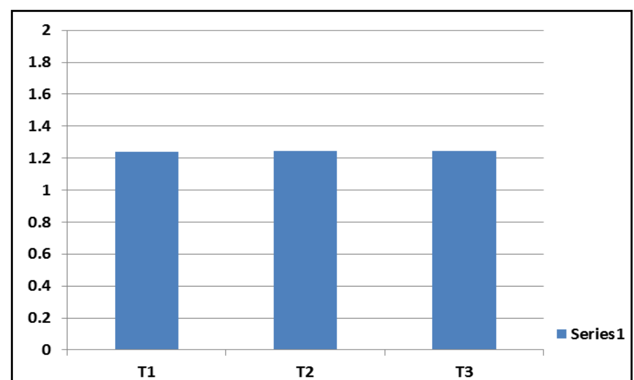

Fig 2: Ocimum basilicum

The graphical representation of Vit. C (ascorbic acid)

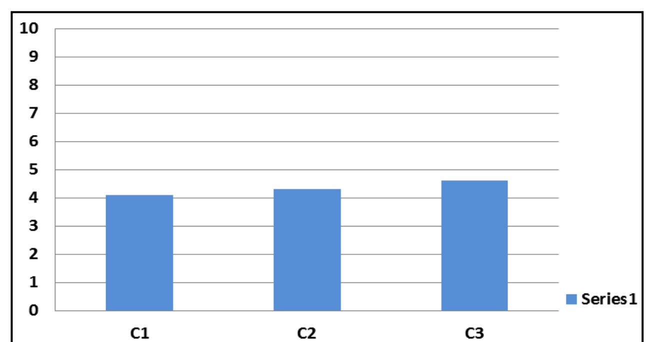

Fig 3: Murraya koenigii

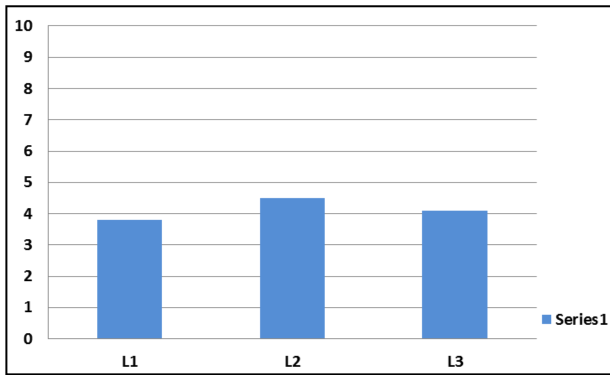

Fig 4: Ocimum basilicum

The graphical representation of DPPH:

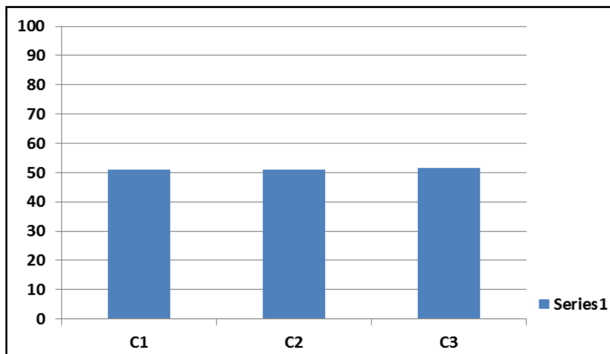

Fig 5: Murraya koenigii

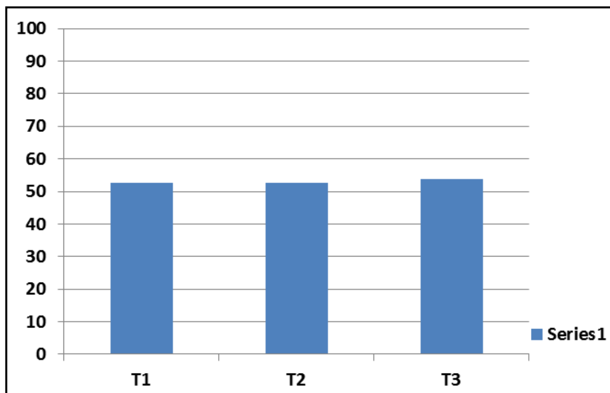

Fig 6: Ocimum basilicum

The graphical representation of moisture content

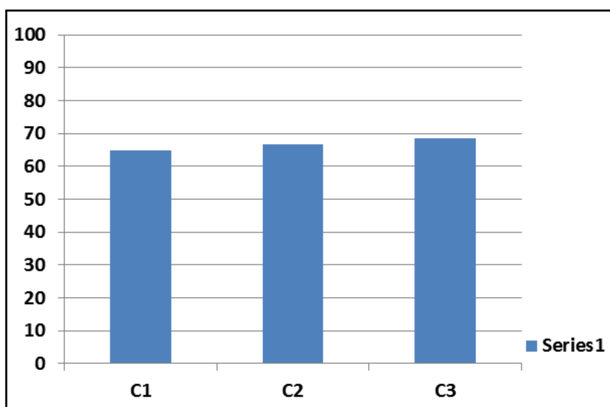

Fig 7: Murraya koenigii

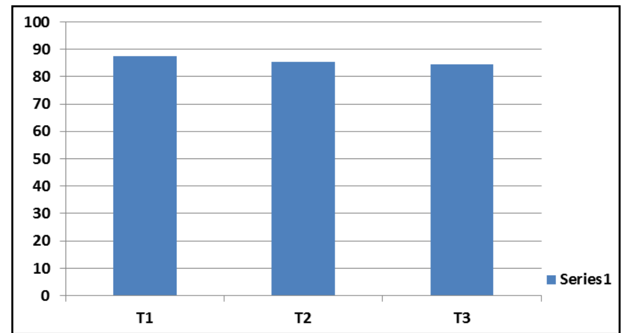

Fig 8: Ocimum basilicum

The graphical representation of ash content

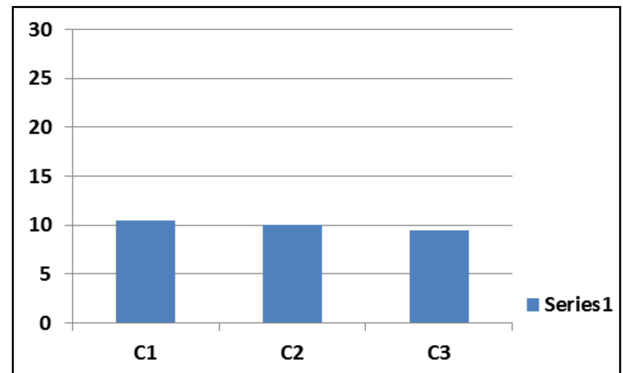

Fig 9: Murraya koenigii

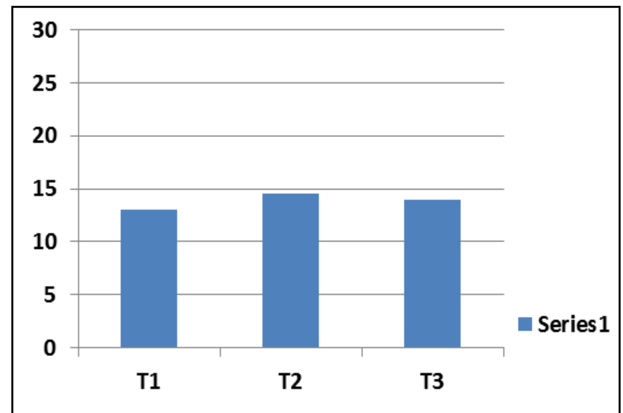

Fig 10: *Ocimum basilicum*

Conclusion

Murraya koenigii and Ocimum basilicum leaves shows the moisture content in leaves ranges respectively from 65-67% and 85-87%% which shows high moisture in ocimum basilicum leaves and ash content was determined 9.5-11% and 14.5-15.5% TPC (Total phenolic content) 1.266-1.269 mg in GAE/g which determines good content in phenolics and ascorbic acid was investigated 4-4.5 and 3-4.5 mg which is almost equal in both leaves and DPPH was determined 50-52% and 52-53% respectively.

The analysis needed to be done for the study of antioxidants and phenolic content in curry and tulsi leaves was done and the results shows that the leaves are fully loaded with antioxidants and phenolic properties and they are enriched as herbal leaves and can be used in every field like herbal food products, medicines, immunity boosters and as food supplements.

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