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Research Article

EXTRACTION AND DETERMINATION OF ANTIOXIDANTS IN MANGO AND LEMON LEAVES

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ABSTRACT

Leaves have various biological effective compounds, they work as anti-natural oxidative stress such as tannins phenolic acids, and have ability to attack radical free. They can be used in many industry such as in pharmaceutical, nutraceuticals, and also in food industry as in food supplement. leaves used as medicinal herbs have various effects on living systems. Some are painkiller, sedatives, anti-inflammatory and antioxidants. The study done on *Magnifera indica* and *Citrus limon* leaves shows the proximate analysis and chemical analysis. and the result of *magnifera indica* and *citrus limon* respectively contains moisture content (69.5% - 75.5% and 54%-58.5%), ash content (14% -15.5% and 14%-15.5%) and ascorbic acid (6.9mg-6.88mg and 4.8mg- 5.5mg) TPC (total phenolic content) (1,266-1.267 mg of GAE/g and 1.267-1.268 mg of GAE/g) and DPPH scavenging activity (63%-65% and 47%-48%).

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INTRODUCTION

Natural phytochemicals presented in herbs are natural bioactive compounds found in plants and these are divided into two groups; primary and secondary compounds have been widely used to cure, and reduce the risk of human diseases (Andarwulan; *et al*; 2012, Do, Q.D; *et al* 2014.) Phytochemicals are highly potential for health benefits due to their inhibition effects and antioxidant activities that has been concerned in a number of diseases (Shahidi *et al*; 2015).

Antioxidant compounds can be found in leaves such as phenolics, carotenoids, anthocyanins, and tocopherols (Jakubowski *et al.*, 1997). Antioxidants are already part of plant materials and supplements. High level of antioxidants present in leaves can defend against free radical damage, leaves contain many valuable compounds that improve the requirement of the human body. (Boots *et al.*, 2008). Different vitamins, and phenol compounds present in plant leaves, act as antioxidant (suffredini *et al* 2004). There are issues about using synthetic antioxidants food preservatives because of their harmful effects on human health such as (BHT) butylated hydroxytoluene and (BHA) butylate hydroxyanisole. Thus, a variety of substitute for these synthetics by various leaves has been formed and discussed.

MATERIALS 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 firm 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 leave sample added 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:

Moisture content (%) = $\frac{w_3 - w_2}{w} \times 100$ Where, w = weight of sample w₂ = weight of petridish + sample w₃ = weight of petridish after drying.

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Ash Content

Ash content was done by the standard method of rangna, 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. Keep the churned crucible in the muffle furnace 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 determined by

(%) Ash content = $\frac{W_3 - W_2}{W} \times 100$ Where, w = sample weight
 W_2 = empty crucible weight W_3 = crucible weight 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. Metaphosphoric acid (6%): 6% metaphosphoric acids were prepared by dissolving 60gm in distilled water and make the volume 1000ml. Standard ascorbic solution: Standard vitamin C solution were prepared by dissolving 10m L-Ascorbic acid in 6% metaphosphoric acid solution and make the volume 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, (suppose it x ml). then again titrate the 20ml of sample solution against dye solution and record the volume of dye used.(suppose it y ml). Amount of ascorbic acid in 100ml of undiluted extract was determined by using the formula.

Ascorbic acid (Vit. C) (mg/100ml) = $(y/x) \times 10\text{mg}$

(TPC) Total Phenolic contents: TPC was measured by the method of Makkar, H.P.S., *et.al*, 2007. Reagents Standard Gallic acid solution: Standard Gallic solution was prepared by taking 50mg Gallic acid and volume make up to 100 ml with distilled water. Folin-Ciocalteu solution: 10% Folin-Ciocalteu solution was prepared 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 of juice sample 0.5ml folin-ciocalteu was added and 2.5 ml Na_2CO_3 (20%) was mixed in test tubes after vortexing the mixture by using vortex shaker and the test tubes were placed 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 make up the volume upto 1ml with distilled water and after this process add 0.5 ml of folin-ciocalteu in the test tube

and then add 2.5 ml (20%) sodium carbonate in each test tube and keep the test tube in dark place for 40-45 minutes after that 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). 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 solution 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 different test tubes respectively. Then 4ml of 0.004% DPPH was mixed to each sample concentrations in the test tubes and then incubate the sample for 30 mins at room temperature in dark. After 30mins incubation, the absorbance was considered at 517 nm using a UV Visible spectrophotometer.

Inhibition activity (IA%) of free radical DPPH percentage was determined using the following correlation:

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

Observation and Results

After all the test performed the following results were analysed and they are presented in graphical representation as following. The graphical representation of moisture content

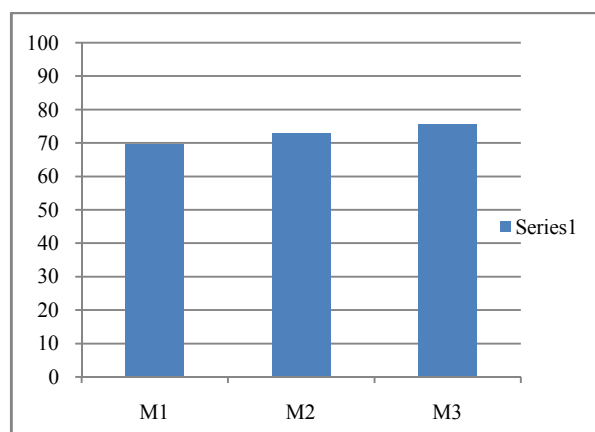


Fig 1 Magnifera indica

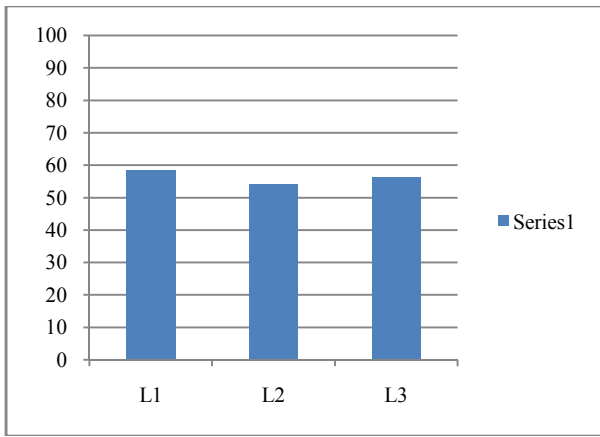


Fig 2 Citrus limon

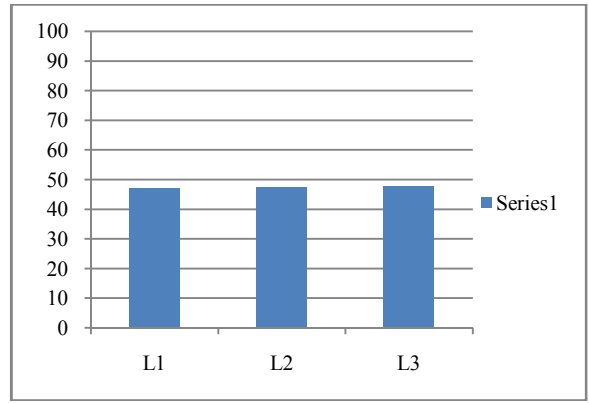


Fig 2 Citrus limon

The graphical representation of ash content.

The graphical representation of vitamin C (Ascorbic acid)

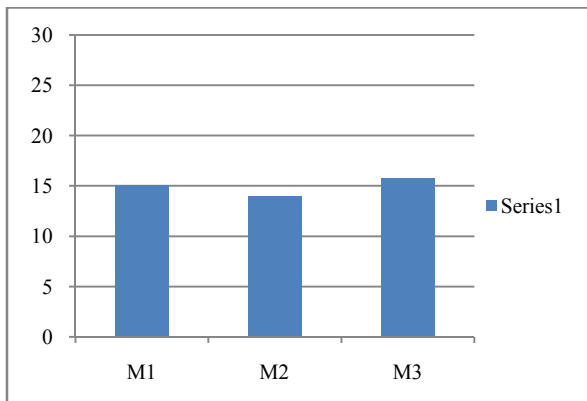


Fig 1 Magnifera indica

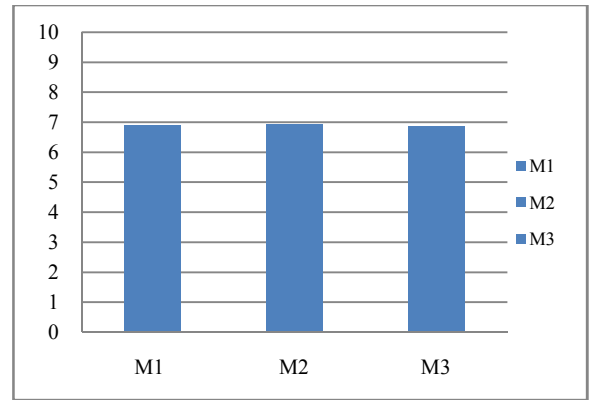


Fig 1 Magnifera indica

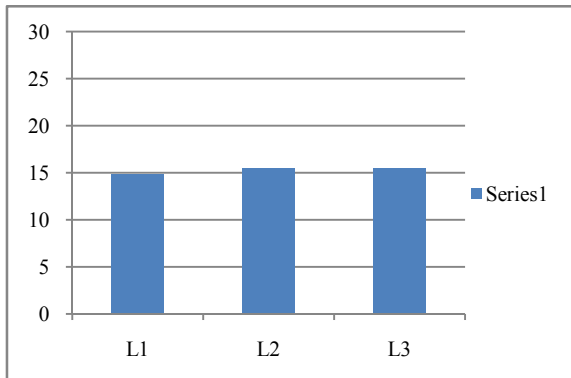


Fig 2 Citrus limon

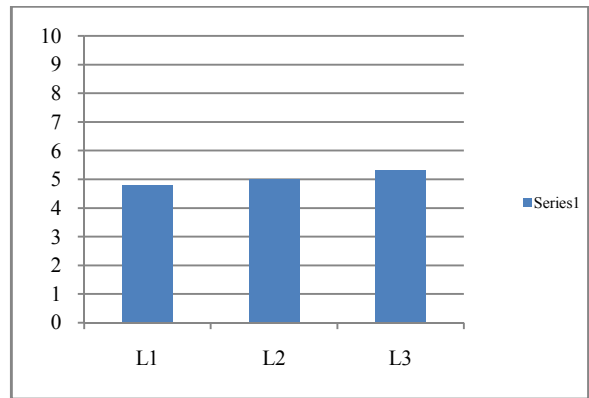


Fig 2 Citrus limon

The graphical representation of DPPH

The graphical representation of TPC (Total phenolic content).

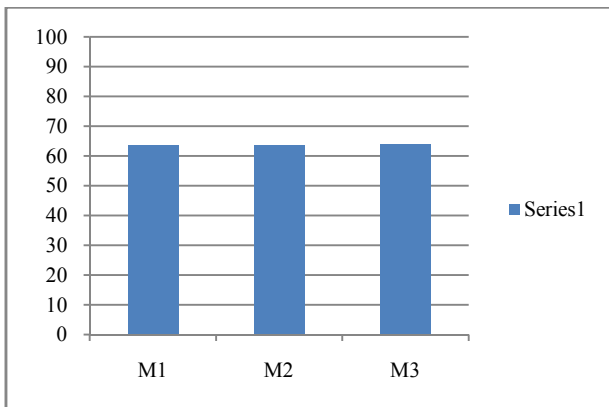


Fig 1 Magnifera indica

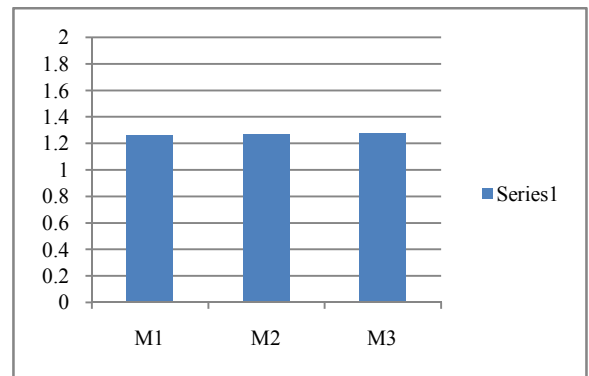


Fig 1 Magnifera indica

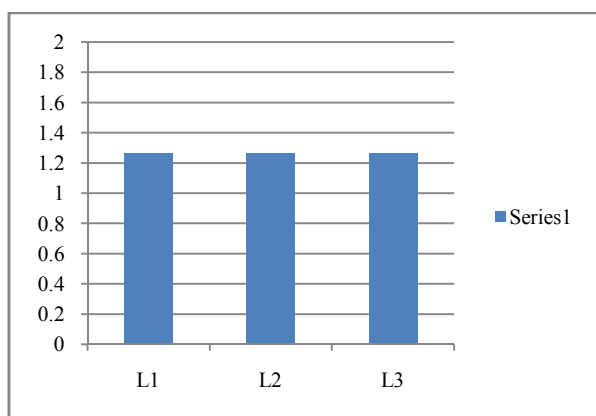


Fig 2 Citrus limon

RESULTS AND DISCUSSION

Magnifera indica and citrus limon leaves shows that the moisture content in leaves ranges respectively 69.5 -75.5 % and 54-58.5% which shows magnifera indica has high moisture content. Ash content was determined 14-15% in both the leaves. TPC (Total phenolic content) was estimated 1.266-1.268 mg in GAE/g which is almost equal and justify that leaves contains good amount of phenolics, ascorbic acid was determined 6.8-6.9 and 4.8-5.5mg which justify vitamin C in higher amount in magnifera indica leaves. DPPH in both the leaves was determined 63-65% and 47-48% which justify that magnifera indica leaves contains more antioxidant activity. (Upadhyay S *et al*)

After the test performed on the leaves we found that the leaves are enriched and loaded with good nutrients and the antioxidants properties required and can be further used in food supplements, in nutraceuticals industry and in many other fields as per the requirement of consumer.

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