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Comparative Analysis of Antioxidant Properties of Curry Leaves and *Euphorbia helioscopia*.

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ABSTRACT

The presence of natural antioxidants in plants was analysed. In vitro antioxidant properties and phenolic content of the extracts of curry leaves and euphorbia were evaluated using various assays. Analysis carried out were total phenolic content, DPPH assay and total flavonoid content. From the analysis curry leaves had the higher yield extraction, higher total phenolic content, antioxidant property and flavonoid content. Total phenolic content has positive correlation with antioxidant capacity. This shows that the plants, especially curry leaves may be potent source of natural antioxidants.

Keywords: Antioxidative activities, Total Phenolic Content, DPPH, Total Flavonoid Content

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INTRODUCTION

Curry leaves- one of the highly popular herbs used in India. Curry leaves represent main portion of Indian cooking style where many of the contemporary recipes are incomplete without curry leaves. Curry leaves are added for the flavour that they add as well as to provide many benefits from health point of view. The biochemicals found in Curry leaves include caryophyllene, cadinene, cadinol, Sabinene, pinene, phellandrene, terpinene, lauric acid, ocimene, palmitic acid, carbazole alkaloids, humulene, bornyl acetate and bisabolene etc. Whereas, Euphorbia is a flowering plant with numerous sub-species. Most of its species are called splurges, which can be herbaceous or succulent. Almost all parts of the plant are of medicinal value. Macro minerals, namely, sodium, potassium, calcium, and lithium have been detected in euphorbia. The plant also contains beta-carotene, vitamin C, and phenolics. Euphorbia is widely used to clear up the respiratory tract and for the treatment of asthma. It has a broncho-dilatory effect that helps to relieve the symptoms of most respiratory diseases. The root of the plant also possesses anti-emetic properties and can combat vomiting.

In recent years much attention has been devoted to natural antioxidant and their association with health benefits. It produces various antioxidative [1] compounds to counteract reactive oxygen species (ROS) in order to survive. ROS, which include free radicals such as superoxide anion radicals (O^{2-}), hydroxyl radicals (OH-) and non-free radicals species such as H_2O_2 and singled oxygen, are various forms of activated oxygen. These molecules are exacerbating factors and cellular injury and aging process. The antioxidant activity of phenolics [2] is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers.

MATERIALS AND METHODS

Plant material

Curry (*M. koenigii L.*) and Euphorbia leaves were obtained from the nursery maintained by VIT University, Tamil Nadu, India. One hundred grams of curry leaves and Euphorbia were dried at ambient temperature for 15–20 days. After complete drying, the leaves were grounded into a fine powder using a domestic electric grinder.

Preparation of leaves extract

The powdered leaves of *Euphorbia helioscopia* plants (100gram) and Curry leaves (*M. koenigii L.*) were primarily extracted[9-11] in Soxhlet extractor by hexane for 6 hours at 65°C in order to remove fatty materials. 25 grams of defatted powders of each plant part material were then individually re-extracted in Soxhlet apparatus for 12 hours at 60°C by 250ml of solvent (ethanol). Following the extractions, solvents were removed using Hot Air Oven maintained at 180°C. Resulting crude extracts were collected into small dark sterile vials and stored at 4°C.

Determination of total phenolic content

The total phenolic contents were determined using the Folin-Ciocalteu [3] reagent as described by Mc Donalds, 50 mg of each extract was thoroughly mixed with 0.5 ml of Folin Ciocalteu reagent and 75 ml of deionized water .This mixture was kept for 10 mins at room temperature and 20% of sodium carbonate (w/v, 1.5 ml) was added to it. The mixture was heated in water bath at 40^{o}C for 20 mins then cooled over an ice bath. The absorbance of all the samples was measured at 755nm with a UV-visible spectrophotometer. Total phenolics were quantified based on standard curve prepared using various concentrations of gallic acid (prepared in 99% ethanol) and expressed as mg of gallic acid equivalent per gram of dry weight (mg GAE/g dry weight). All samples were analysed in triplicates.

Antioxidant activity using DPPH assay

The antioxidant activity [4-7] was investigated using the stable free radical 1, 1 diphenyl-2-picrylhydrazyl (DPPH) assay, which estimated the hydrogen donating or the radical scavenging ability of the examined extract. When reacting with an antioxidant compound, the DPPH solution, initially purple in color,

changed to yellow. The degree of discoloration denotes the scavenging potency of the tested compound which can be measured spectrophotometrically at 517nm. The antioxidant activity of Euphorbia helioscopia leaves and Curry leaves extracts were monitored according to the method in which 2 mg of DPPH was dissolved in 100 ml of ethanol (control).A blank containing 100ml of ethanol was taken in another test tube. Then, 1 ml of extract and 1 ml of the control were taken in another test tube and incubated for half an hour. The mixture was then allowed to stand at room temperature in the dark for one hour. Absorbance of the mixture was measured at 517nm by an UV-Visible spectrophotometer. Inhibition of free radical DPPH in percentage terms was calculated using the following equation: % of inhibited DPPH= ((AC517-AE517)/ AC517) x100), Where AC517 is the absorbance of the control and AE517 is the absorbance of the plant extract after 1 hour incubation.

Determination of total flavonoid content

The total flavonoids [8] contents were determined by colorimetric method and expressed as mg (QE) quercetin equivalent per g of dry weight, using the method described by Chang et al., 0.5 ml of each plant extracts (prepared from 1mg of crude extract dissolved in 1ml of ethanol) were mixed with water (diluted) 4ml in 10 ml volumetric flask. Then 5% $NaNO_2$ (0.3 ml) was added to the flask kept at room temperature for 5 minutes. This was further supplemented with 10% $AlCl_3$ (0.3 ml), kept for 6 minutes at room temperature, 1.0M NaOH (2ml) and water 2.4 ml were added to the reaction flask and mixed well. The resulting mixtures were mixed well and incubated for 30 minutes at obscurity. The absorbance of the reaction mixture was measured at 510 nm with a UV-visible spectrophotometer. Samples were analysed in triplicates.

Chemicals, Reagents and Apparatus

Chemicals and reagents used in all the three experiments are as follows: folin-Ciocalteu reagent, deionized water, 20% Sodium carbonate, Ethanol, DPPH, Water, 5% NaNO2, 10% $AlCl_3$, 1.0M NaOH, Soxhlet Apparatus, Hot Air Oven, UV-Visible Spectrophotometer and Incubator are used.

RESULTS AND DISCUSSION

Total Phenolic Content

The Total Phenolic Content of the Curry and Euphorbia leaves extracts are presented below. The higher value was obtained for curry extract *i.e* 24.44 mg/g GAE and the lower value of 14.98 mg/g GAE was observed in the case of euphorbia leaves extract with ethanol as the solvent.

DPPH Assay

The free radical scavenging activity is depicted in graph (2). Curry leaves extract at a concentration of 0.02mg DPPH/ml ethanol exhibited the free radical scavenging activity of 81.13% whereas the inhibition value of 17.41 % was determined when the ethanol extract of euphorbia leaves were used.

Total Flavonoid Content

The Total Flavonoid Content obtained for the curry and euphorbia leaves samples were 5.2qe and 3.88qe respectively. The higher value is again observable in the case of curry leaves which are responsible for its antioxidant property.

Figure 1: Soxhlet Apparatus

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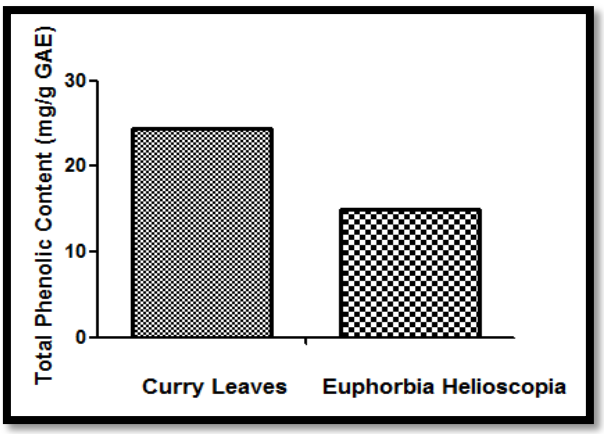
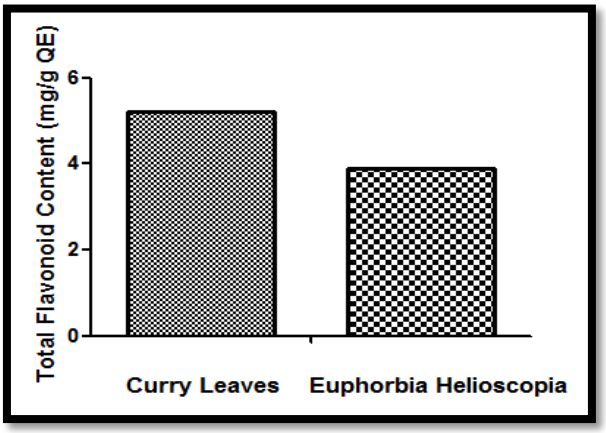


(a)



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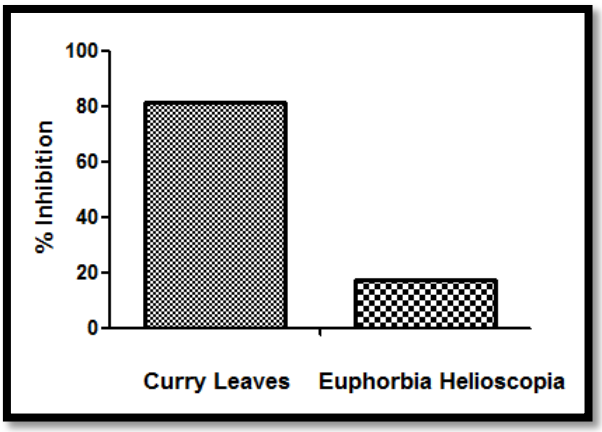

(c)

Figure 2: a) Total Phenolic Content b) Flavonoid Analysis c) DPPH Assay

Table 1: Total Amount Of Phenolic And Flavonoid Content Of Extract Of Curry Leaves And Euphorbia Leaves

Extract	Total phenolics mg/g plant extract (in GAE)	Total flavonoid mg/g plant extract (in QE)
CURRY LEAVES	24.44 ± 0.12	5.2 ± 0.31
EUPHORBIA LEAVES	14.98 ± 0.08	3.88 ± 0.59

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CONCLUSIONS

The results of this study showed that the higher antioxidant activity, total phenolic content and total flavonoid content were exhibited by curry leaves extracts via extraction with ethanol as solvent in comparison with that of the euphorbia. The ethyl alcohol extract of curry leaves prove to be a very good lead for extraction of an effective natural nutraceutical or antioxidant drug. Thus the studied natural concentrates can be recommended as suitable substitutes for synthetic antioxidants.

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