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Correlation of TPC and TFC with Antioxidant Activity of Selected Indian Medicinal Plants

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ABSTRACT

In recent times, interest has focused on phytochemicals as new sources of natural antioxidants. Therefore, the methanolic crude extracts of *Acorus calamus*, *Lantana camara*, *Plumbago zeylanica* and *clitoria ternatea*, were screened for total phenols, flavonoids, and free radical scavenging activity. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). Significant differences in DPPH scavenging activity were found between the species investigated, ranging from 26.36 ± 1.00% to 91.14 ± 1.02%. The highest radical scavenging activity was observed in *Acorus calamus* (91.14 ±1.02% inhibition). The total phenol content of the investigated species ranged from 23.43±0.18 to 57.33± 2.21 mg GAE/g extract, while flavonoid content ranged from 5.76±2.12 to 20.17 ± 2.72 mg QE/g extract. A weak linear correlation between total phenolic or flavonoid content and antioxidant activity was found (correlation coefficient, R^2 =0.2319 and R^2 = 0.2605, respectively).

Key Words: *Acorus calamus, Lantana camara, Plumbago zeylanica* and *Clitoria ternatea,* phenolic content, flavonoid content, antioxidant

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INTRODUCTION

Medicinal plants play a essential role in the health care of ancient and modern cultures. Ayurveda, the Indian system of medicine mainly uses plant based drugs or formulations to treat various human ailments because they contain the components of therapeutic value[1]. In addition, plant based drugs remain an important source of therapeutic agents because of the availability, relatively cheaper cost and non-toxic nature when compared to modern medicine[2]. Many herbs contain antioxidant compounds which protects the cells against the damaging effects of reactive oxygen species. Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non nutrient molecules, many of which display antioxidant properties and can protect the human body against cellular oxidation reactions[3]. It is important to screen different types of medicinal plants for their antioxidant potential.

Natural antioxidants such as phenols, flavonoids and tannins are increasingly attracting because they are natural disease preventing, health promoting and anti-ageing substances[4]. Antioxidants may serve the task of reducing oxidative damage in humans induced by free radicals and reactive oxygen species under oxidative stress conditions. These conditions can cause DNA and protein damage, lipid peroxidation, cancer, ageing and inflammatory activity[5]. Phenolics are an important class of secondary plant metabolites possessing an impressive array of pharmacological activity. One of the more prominent properties of the phenolics is their excellent radical scavenging ability. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes[6]. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity[7]. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases[8].

There is currently enormous interest in natural antioxidants and their role in human health and nutrition[9]. Considerable amount of data have been generated on antioxidant properties of medicinal plants around the globe[10,11]. However, traditionally used medicinal plants await such screening. On the other hand, the medicinal properties of plants have also been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability[12]. Some of these plants have shown potent antioxidant activity[13]. However, majority of plants have not yet been screened for

such activity. So, in order to contribute further to the knowledge of Indian medicinal plants, our present study is focussed on four plants namely *Acorus calamus, Lantana camara, Plumbago zeylanica and Clitoria ternatea* to determine their antioxidant and free radical scavenging properties. The literature survey showed scanty information available on these plants and thus prompted us to analyze these medicinal plants. Further an attempt has also been made to find the correlation between phenolic content, Flavonoid content and antioxidant activity of these plants.

MATERIALS AND METHODS

Standards and reagents

Folin-Ciocalteau reagent, Aluminum chloride,Potassium SodiumL-(+)-Tartrate Tetrahdrate, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were used as reagents. All chemicals and solvents were of analytical grade.

Preparation of extracts

Four medicinal plants studied were collected during the period of August to September from *Sanjivani Ayurvedic Nursery Bhopal.* All the plant materials were further identified in the Department of Botany, SNGGPG College Bhopal, (India). The plant material was cleaned and air-dried in shed at room temperature (26°C) for 2 weeks, after which it was grinded to a uniform powder. A quantity (100g) of powdered plants material was weighed and subjected to soxhlation with methanol for 96 hrs. The solvents was distilled off at lower temperature under reduced pressure and concentrated to dryness (crude extract). The dried extract was weighed and then stored in a freezer. The crude extract was used for the experiments.

Total Phenolic content (TPC)

Folin-Ciocalteu reagent assay is popular for quantifying total phenolics. This method is based on reducing power of phenolic hydroxyl groups, which react with Folin-Ciocalteu phenol reagents to form chromogens that can detected spectrophotometrically.

TPC is measure by spectrophotometry, using gallic acid as standard, according to the method described by the International Organization for Standardization (ISO)[14]. In brief, 0.5 ml of extract and 0.1 ml (0.5 N) Folin-Ciocalteu's reagent was mixed and the mixture was incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate solution was added and further incubated for 30 min. at room temperature and the absorbance was measured at 750 nm. A standard calibration plot was generated at 750nm using known concentrations of gallic acid. Total phenol values are expressed in terms of gallic acid equivalent per gm of sample.

Total flavonoid content (TFC)

Total flavonoid contents were measured by Aluminum chloride colorimetric assay[15]. Methanolic

extracts that has been adjusted to come under the linearity range and different dilution of standard solution of Quercetin (10-100µg/ml) were added to 3ml of water. To the above mixture, 0.1ml of 5% $C_4H_4O_6KNa.4H_2O$ (Potassium Sodium L-(+)- Tartrate Tetrahdrate) was added. After 5 minutes, 0.1ml of 10% $AlCl_3$ was added and the total volume was made up to 3 ml with distill water. It was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415nm with spectrophotometer[16]. A standard calibration plot was generated at 415nm using known concentrations of quercetin. The concentration of flavanoids in the sample was calculated from the calibration plot and expressed as mg Quercetin equivalent per gm of sample.

Scavenging activity on DPPH radical

This assay was based on the method described by Yen GC, Duh PD (1994)[17] with some modifications. Briefly, 0.04% solution of DPPH in methanol was prepared and it must be protected from light influence by maintaining the dark condition and also fold by aluminum foil and 1ml of this solution was added to 1ml various conc.(200 µg/ml) of extracts or standard solution of (200 µg/ml). Absorbance was taken after 30min at 517nm. Ascorbic acid was used as the reference materials. Methanol only was used as control of experiment. The percentage of inhibition was calculated by comparing the absorbance values of the test samples with those of the controls (not treated with extract). The inhibition percentage was calculated as radical scavenging activity as follows:

$$\% \text{ Scavenging Activity} = \frac{\text{Abs. control } (A_0) - \text{Abs. sample } (A_1)}{\text{Abs. control } (A_0)} * 100$$

Where, A_0 control = absorbance of control

A_1 sample = absorbance of test or STD taken as Ascorbic acid.

RESULTS AND DISCUSSION

There is an increasing evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in medicinal plants[18,19]. The details of four medicinal plants selected in this study are described in table 1

DPPH assay

The antioxidant activity of medicinal plants is mainly related to their bioactive compounds, such as phenolics and flavonoids. In this study, the antioxidant capacity of four methanolic extracts of medicinal plants was systematically evaluated. The DPPH inhibition of different plant extracts is

summarized in Table 2. Significant differences in DPPH scavenging activity were found between the four plants investigated, ranging from 26.36 ± 1.00% to 91.14 ± 1.02%. This wide range of antioxidant activity may be attributable to the wide variety of bioactive compounds, such as phenolics, flavonols, carotenoids, and tannins, present in the selected medicinal plants.

Table 1: Ethnobotanical details of the selected Indian medicinal plants

Plant name (Family)	Common Name	Part Used	Traditional Uses
Acorus calamus (Acoraceae)	Bach	Root	Stomachic, dyspepsia, colic, remittent fevers, nerve tonic, in bronchitis, dysentery in children[20]
Lantana camara (Verbenaceae)	Lantana weed	Leaves	Leaves were used to treat cuts, ulcers, intestinal worms and rheumatism[21]
Plumbago zeylanica (Plumbaginaceae)	Chitra	Root	Used in paralytic affection, secondary syphilis, leprosy and Opthalamia[22]
Clitoria ternatea (Fabaceae)	Aparajita	Leaves	used as diuretics, antihelmintic, antidiabetic, antipyretic and brain tonic [23]

Table-2. Total phenolic content (TPC), Total flavonoid content (TFC) and Antioxidant activity of four plant extracts.

Plants extracts	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	% inhibition of DPPH
Acorus calamus	23.43±0.18	8.16±2.32	91.14±1.02
Lantana camara	57.33± 2.21	20.17±2.72	80.00±1.34
Plumbago zeylanica	42.14±1.10	14.87±2.45	75.00±1.02
clitoria ternatea	18.26±1.65	5.76±2.12	26.36± 1.00

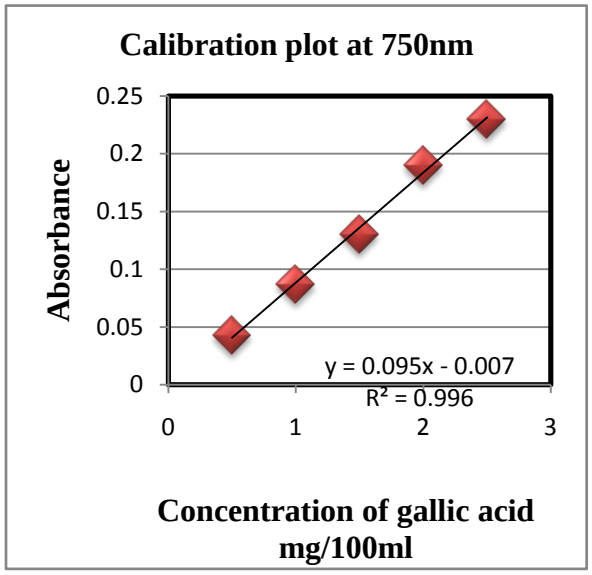
Values are mean (n = 3) ± SD (standard deviation).

Total phenolic content

Phenolics are well established to show antioxidant activity and contribute to human health. In this study, the total phenolic content was determined using the Folin– Ciocalteu method, with gallic acid as a standard. The content of phenolics was evaluated from the regression equation of the calibration curve (R^2 = 0.9963, y = 0.0954x+ 0.0071), expressed in GAE as milligrams per gram of extract (mg GAE/g extract). The content of phenolics varied from 18.26±1.65 to 57.33± 2.21 mg GAE/g extract (Table 2).

Total flavonoid content

Flavonoids are well-known antioxidant constituents of plants and possess a broad spectrum of chemical and biological activity, including radical scavenging properties[24]. Therefore, the total content of flavonoids was evaluated from the regression equation of the calibration curve (R^2 = 0.9891, y =0.0135x –0.0026), expressed in QE as milligrams per gram of extract (mg QE/g extract). The content of flavonoids varied from 5.76±2.12 to 20.17±2.72 mg QE/g extract (Table2).



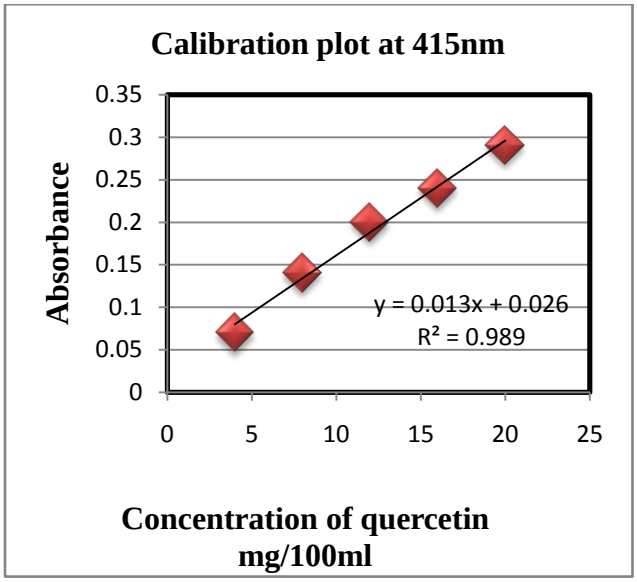
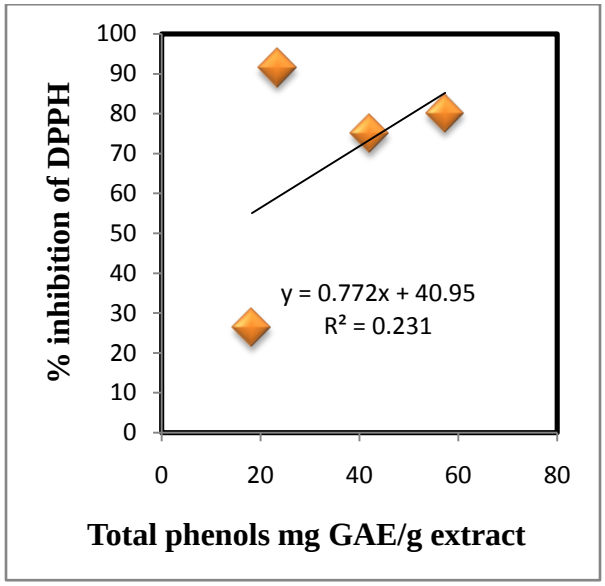


Figure-1: Calibration plot for phenolics **Figure-2: Calibration plot for flavonoids**

Correlation between antioxidants and total phenolics and total flavonoids

The total phenolic content of the investigated plants extracts showed a weak correlation with antiradical activity (Figure 1; R^2 = 0.2319, y = 0.0772x + 40.995). This low correlation between total phenols and DPPH scavenging activity suggests that the major antioxidant components might not be phenolics, and could be sterols, tocopherols, ascorbic acid, and carotenoids. The correlation between total flavonoids and DPPH scavenging (R^2 = 0.2605, y = 2.2408x + 40.77) was also very weak (Figure 2), consistent with the findings of Imeh and Khokhar[25], who reported a weak correlation in fruits, but unlike the results of Kahkonen[26], who did not find such a weak correlation. The weak relationship between antioxidant activity and total phenolic compounds in this report may be caused by other factors; for example, flavonoids with a certain structure and hydroxyl position in the molecule can only act as proton donors and show radical scavenging activity[27]. other phytocontituents present in crude extracts which affects the results. Moreover, measurement of phenolics using the Folin–Ciocalteu method might not be a good indicator of antioxidant capacity because this assay estimates total phenolics present in the extract, but is subject to interference, giving rise to elevated apparent phenolic concentrations[28]. In addition, the antioxidant activity of plant extracts is not limited to phenolics, but also includes vitamins C and E, carotenoids, and chlorophylls[29]. Moreover, significant differences in total phenolics can be attributable to extraction methods, time of collecting samples, environment, and genetic differences between tested samples[30]. On other hand several studies have reported a high correlation between phenolic content and antioxidant activity[31].



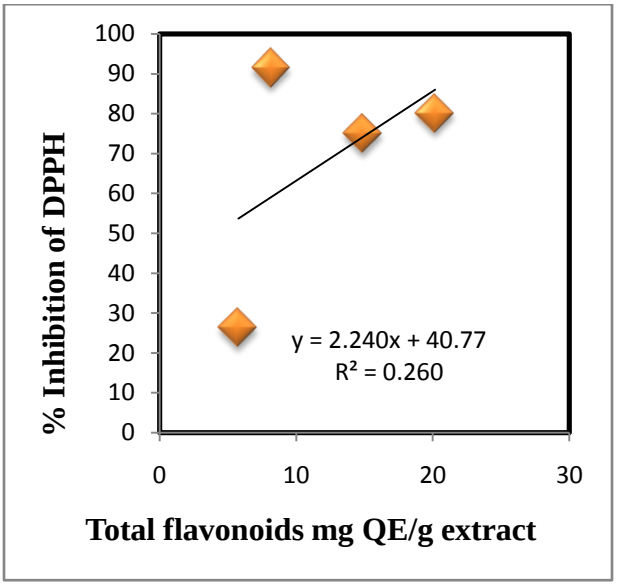


Figure 3, 4. Linear correlation between the antioxidant activity and total phenolic content and total flavonoid content of the methanol extracts of four medicinal plants.

CONCLUSION

In conclusion, four Indian medicinal plant extracts in this research exhibited different degrees of antioxidant activity. In particular, *Acorus calamus, Lantana camara and Plumbago zeylanica* can be considered as promising sources of natural antioxidants and as possible preventative agents of some common human health disorders. However, the total phenolic and flavonoid content showed a weak correlation with the antioxidant activity of the investigated plant. Hence, detailed studies on the role of individual phytochemicals involved in the antioxidant activity of specific plants are required for their use as medicinal plants and in the pharmaceutical industry.

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