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Correlation of antioxidant activity and phytochemical profile in native plants

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Abstract

Purpose – The purpose of this paper is to study the structure-activity correlation of four medicinal plants – *Ocimum basilicum* L., *Piper betle* L., *Grewia asiatica* L., *Lantana camara* L. in crude methanolic extract. These plants have been used in Ayurvedic preparation as alternative medicine. The contents of phenolic compounds (flavonoids, anthocyanin) and antioxidant capacities were evaluated.

Design/methodology/approach – Folin-Ciocalteu method was used for the analysis of the phenolic compounds. Total flavonoids were also evaluated. DPPH, ABTS +, FRAP and H_2O_2 assays were used to evaluate antioxidant activity. Most of the assays were determined spectrometrically.

Findings – *O. basilicum* L. had the highest of total phenolics content (305.11 mg GAE/g), highest content of anthocyanins (15.427 mg/Kg) and total flavonoids (9.692 QE mg/g). The DPPH, ABTS +, FRAP and H_2O_2 assay indicated that these plants possessed considerable antioxidant activities.

Originality/value – The results also showed that *O. basilicum* and L. *camara* from red colored cultivars possessed high contents of phenolic compounds. FRAP assay showed AOA in the following sequence – *Lantana camara* > *Ocimum basilicum* > *Piper betle* > *Grewia asiatica*. Since L. *camara* showed highest antioxidant capacity thus it can be a potential resource for commercial antioxidant.

Keywords India, Medicinal plants, Alternative medicine, Grewia asiatica, Lantana camara, Ocimum basilicum, Piper betle, Antioxidant activity, DPPH, FRAP, ABTS

Paper type Research paper

1. Introduction

Four plant species *Ocimum basilicum*, *Piper betle*, *Grewia asiatica*, *Lantana camara*, were selected to carry out phytochemical analysis along with their antioxidant activity (AOA). *G. asiatica* (Phalsa, *Tiliaceae*), is a warm climate fruit plant of North India. The fruit is believed to have astringent and stomachic properties, is known to have anti malaria and anti ulcer effects due to presence of flavonoids and tannins (Gupta *et al.*, 2010). It alleviates inflammation and is administered in respiratory, cardiac and blood disorders, as well as in fever.

P. betle (Betel leaf, *Piperaceae*), the chief constituent of the leaves is a volatile oil containing phenolic compounds. Its leaves yield an aromatic essential oil, rich in powerful antiseptic and are also rich in starch, sugars and tannins. The betel leaf essential oil is valued in Ayurveda as being stimulating, carminative, aromatic, warming, antiseptic and even as an aphrodisiac, being especially used to harden the gums, preserve the teeth, sweeten the breath and improve the voice.

L. camara (*Verbenaceae*) is native of tropical and subtropical India. The plant has been used in folk medicine, in many parts of the world, to treat fever, influenza, asthma, bronchitis cancers, tumours and a variety of other disorders. Decoctions were applied

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externally for leprosy and scabies. It has been claimed that a steroid, lancamarone, from the leaves exhibited cardiotonic properties (Sharma and Kaul, 1959).

O. basilicum L. (Basil, *Lamiaceae*) has a number of phenolic compounds with strong AOA. It is an aromatic herb that has been used traditionally as a medicinal herb in the treatment of headaches, coughs and other ailments and as insect-controlling agent (Grayer *et al.*, 1996). Chemical studies revealed that *O. basilicum* has major component as rosmarinic acid is the predominant phenolic acid present in both flower and leaf tissues and has been an integral part of traditional Iranian medicines (Javanmardi *et al.*, 2002).

Several types of plant materials, such as vegetables, fruits, seeds, hulls, wood, bark, roots and leaves, etc. have been examined as potential sources of antioxidant compounds (Velioglu *et al.*, 1998; Vankar and Srivastava, 2008; Vankar *et al.*, 2009).

Phenolic compounds are widely distributed in plants, which have gained much attention, due to their antioxidant activities and free radical-scavenging abilities, which potentially have beneficial implications for human health (Govindarajan *et al.*, 2007). Flavonoids and other polyphenols possess, anti-allergic, anti-platelet, anti-ischemic, and anti-inflammatory activities, among others, and most of these effects are believed to be due to the antioxidant capacity.

All the chosen four species possess essential oil or as fatty acids. It is well known from the literature that essential oil (containing terpenoids) also have AOA.

2. Materials and methods

2.1 Chemical and reagents

All the chemicals and reagents used were of analytical grade.

2.2 Plant materials

All samples were further identified by Dr K. Haridasan, former Systematic Botanist of the State Forest Research Institute, Dept. of Environment and Forests, Govt. of Arunachal Pradesh, Itanagar, India.

2.3 Extraction

Respective plant parts were dried in an oven at 60°C for 1 hour to remove excess water content and finely chopped. All plants were weighed 100 g and transferred into a filter paper extraction thimble and inserted into a 500 ml reflux flask, then extracted with 400 ml absolute methanol for about 6 hour in a Soxhlet apparatus. After Soxhlet extraction, the extract was filtered through Whatman No. 42 filter paper for removal of fibrous particles and extracts was concentrated using rotary vacuum evaporator at 60°C. After that solvent was removed completely in *vacuo*. The mass of the remaining plant extract was measured and they were used for the phytochemical analysis.

2.4 Determination of total phenolic content-Folin method

The total phenols in the methanol solvent extracts of plants were determined using Folin-Ciocalteu (FC) reagent (Taga *et al.*, 1984). 0.1 g of the dry extract was leached with 100 ml of DW and filtered. To the 4.5 ml of DW, 0.5 ml filtrate, 0.2 ml of 2N FC reagent, 0.5 ml of 2.5 percent sodium carbonate and finally 4.3 ml of de-ionized water were added and mixed completely. After 30 min, the absorbance of the solution at 725 nm was measured. Quantification was based on the standard curve of gallic

acid (0-0.5 mg/ml), and expressed as gallic acid equivalent (GAE)/100 g extract using the following linear equation based on the calibration curve as shown in equation (1):

$$A = 0.0011 \times C^2 + 0.0656 \times C + 0.001 \quad \text{Coefficient} = 0.99 \tag{1}$$

where A is the absorbance and C is concentration as GAEs (Figure 1).

2.5 Determination of total flavonoid content – aluminum chloride colorimetric assay method

Total flavonoid contents were measured with the aluminum chloride colorimetric assay. A method (Zhishen *et al.*, 1999) was modified, employed. 0.5 ml aliquot of the plant extracts (10 mg/10 ml), was mixed with 0.4 ml DW, 0.15 ml of 5 percent sodium nitrite ($NaNO_2$) was added and the mixture was allowed to react for 5 min. Following this, 0.15 ml of 10 percent aluminium chloride ($AlCl_3$) was added and the mixture was made to stand for 5 min further. Finally, the reaction mixture was diluted with distilled water and the absorbance at 510 nm was recorded and expressed as mg Quercetin equivalents (Figure 2).

2.6 Determination of total anthocyanin content

Total anthocyanin analysis was performed using a spectrophotometric differential pH method (Rapisarda *et al.*, 2000) with a few modifications. Two lyophilised samples of 500 mg were treated with 10 ml of buffer solution, pH 1.0 (125 ml of 0.2 M potassium chloride and 375 ml of 0.2 M hydrochloride), and 10 ml of buffer solution, pH 4.5 (400 ml of 1 M sodium acetate, 240 ml of 1 M hydrochloride and 360 ml of DW), respectively. The mixture was homogenised and centrifuged twice at 4°C at 5,000 rpm for 15 min. The supernatant was collected and its absorbance was read at 510 nm. Total anthocyanin amount was determined by the following equation (2):

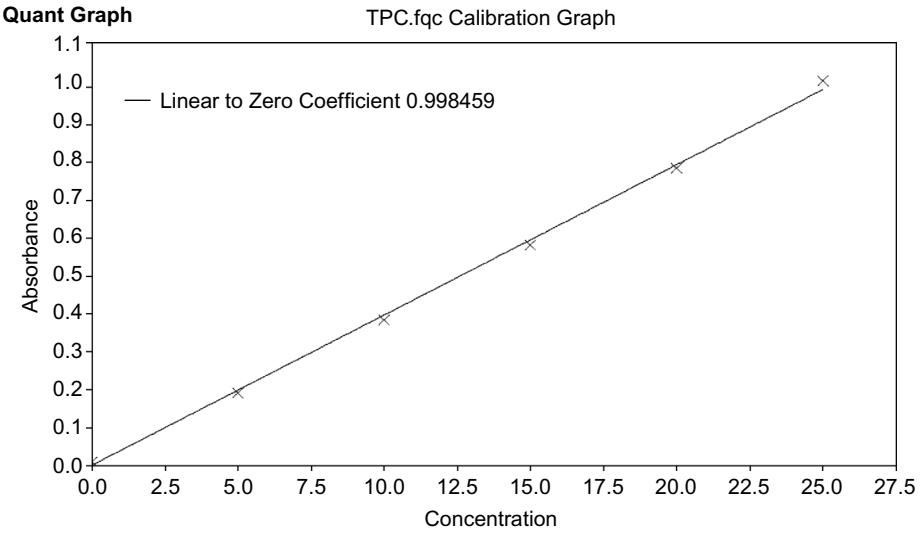

Figure 1.
Calibration graph-total phenol content

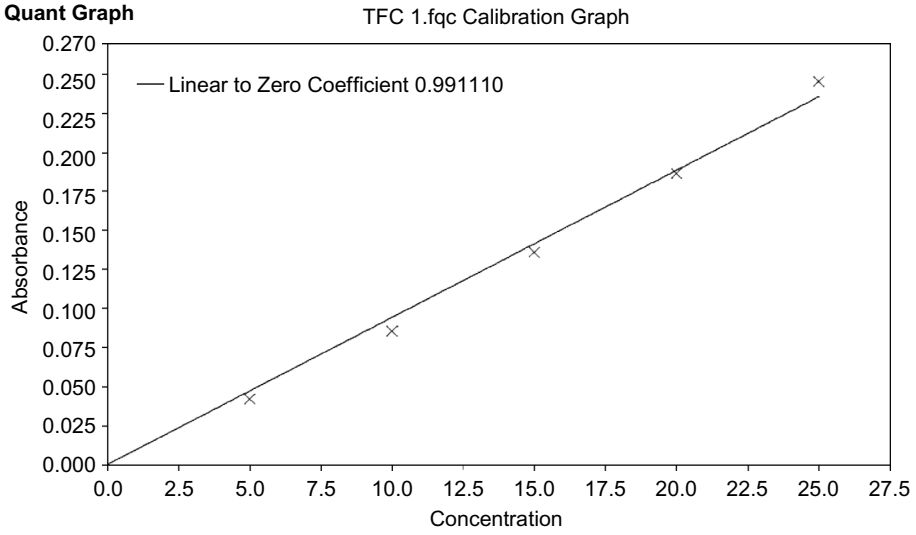

Figure 2.
Calibration graph-total flavonoid content

$$\mathrm{TAC\,(mg/g)} = \frac{\mathrm{A} \times \mathrm{MW} \times \mathrm{DF}}{\varepsilon \times 1} \tag{2}$$

where the terms in parentheses indicate the difference between the absorbance value at 510 nm at pH 1.0 and 4.5 solutions, respectively. 484.8 is the molecular mass of cyanidin-3-glucoside chloride, 24,825 is its molar absorptivity (ε) at 510 nm, and F is the dilution/concentration factor related to the sample weight, the extract volume and the yield of the lyophilisation process.

2.7 Determination AOA

2.7.1 ABTS$^{\cdot+}$ free radical decolorization assay. Antioxidant capacities of the extracts were evaluated using a heλois α spectrophotometer, by the improved ABTS$^{\cdot+}$ method (Re *et al.*, 1999) with slight modification. Briefly, ABTS$^{\cdot+}$ radical cation was generated by a reaction of 7 mM ABTS with 2.45 mM potassium persulfate. The reaction mixture was allowed to stand in the dark for 16 h at room temperature and used within two days. The ABTS$^{\cdot+}$ solution was diluted with ethanol, to give an absorbance of 0.700 ± 0.050 at 734 nm. All samples were diluted appropriately to give absorbance values 20-80 percent of that of the blank. 50 ul of diluted sample were mixed with 1.9 ml of diluted ABTS$^{\cdot+}$ solution. The mixture was allowed to stand for 6 min at room temperature and the absorbance was immediately recorded at 734 nm. The results were expressed as μM Trolox/g dry weight of dry weight and scavenging effect (percent) was calculated as equation (3):

$$\left[\frac{(\mathrm{OD}_{734\mathrm{control}} - \mathrm{OD}_{734\mathrm{sample}})}{(\mathrm{OD}_{734\mathrm{control}})}\right] \times 100\% \tag{3}$$

2.7.2 DPPH free radical scavenging assay. To determine the free radical scavenging activity of the crude extract of plants DPPH (2,2-diphenyl-1-picrylhydrazyl) radical free

radical scavenging assay (Yamaguchi *et al.*, 1998) with minor modification was used. The sample was dissolved in methanol, 3 ml of sample and 1 ml of 0.3 mM DPPH reagent prepared in methanol were vigorously shaken and incubated in the darkness at room temperature for 30 min. After incubation, the absorbance of the reaction mixture was measured in triplicate, spectrophotometrically at 517 nm. The scavenging effect of DPPH free radical was calculated by using equation (4):

$$\text{Scavenging effect}\,(\%) = \left(1 - \frac{\text{Absorbance of sample at 517 nm}}{\text{Absorbance of control at 517 nm}}\right) \times 100 \qquad (4)$$

2.7.3 Determination of AOA in FRAP system. The extracts were suspended in distilled water and mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6), and 2.5 ml of 1 percent $K_3Fe(CN)_6$. The mixture was incubated at 323 K for 20 min, 2.5 ml of 10 percent, trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1 percent), and the absorbance was measured at 700 nm (Oyaizu, 1986) and expressed in terms of gallic acid equivalent (mg g^{-1} of dry mass).

2.7.4 Scavenging of hydrogen peroxide. The ability of extracts to scavenge hydrogen peroxide was determined by little modification (Ruch *et al.*, 1989) here the solution of hydrogen peroxide (100 mM) was prepared instead of 40 mM in phosphate buffer saline of (pH 7.4), at various concentration of aqueous and ethanolic extract (100-1,000 μg/ml) were added to hydrogen peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min. The percentage inhibition activity was calculated from:

$$\%\,\text{Scavenged}\,[H_2O_2] = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100 \qquad (5)$$

where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample.

3. Results and discussion

We carried out phytochemical investigations on *G. asiatica L.* (Phalsa) fruit, *P. betel L.* (Paan) leaves, *O. basilicum L.* (Basil) flowers and *L. camara* (red sage) flowers. The evaluation of total phenolic, total flavonoid, total anthocyanin contents (TACs) and the AOA of the crude methanolic extract were carried out.

3.1 Estimation of total phenolic content

The total phenolic compounds (TPC) values for all extract are shown in Table I. Higher TPC value was observed in the case of *O. basilicum L.* (Basil) extract,

Plant species	TPC (mg GAE/g)	TFC (QE mg/g)	TAC (mg/Kg)
G. asiatica (fruit)	144.11	4.608	4.882
L. camara (flower)	223.14	4.776	2.003
O. basilicum (leaf)	305.11	9.692	15.427
P. betel (leaf)	206.55	2.740	8.104

Table I. Total phenolic and flavonoid content in traditional plants

suggesting that it contains good phenolic contents. The order of TPC is the following: 305.11 GAE mg/g for *O. basilicum*, *L. camara* – 223.14 GAE mg/g, *P. betel* – 206.55 GAE mg/g and *G. asiatica* 144.11 GAE mg/g (Figure 3).

3.2 Estimation of total flavonoid content

Total flavonoid content (TFC) value ranges from 2.74 to 9.692 QE mg/g where *O. basilicum* flower showed remarkably high content of TFC 9.692 QE mg/g, followed by *L. camara* flower, *G. asiatica* fruit and *P. betel* leaves, respectively, (Table I). The results indicate that these extracts contained high polyphenols and flavonoid content.

3.3 Estimation of TAC

TAC in *O. basilicum* showed remarkably high value 15.427 mg/kg which was followed by *P. betel* 8.104 mg/kg, *G. asiatica* 4.882 mg/kg and *L. camara* 2.003 mg/kg. The result of the present study showed that among the different plant species, highest values for flavonoid, anthocyanin and phenolic compounds were found in the leaves of *O. basilicum*.

3.4 AOA of plant extract by four different methods

The antioxidant capacity was measured by four different methods the TEAC, DPPH, FRAP and H_2O_2 as shown in Figure 4 and Table II. *L. camara* showed best results (95.72 percent) while *G. asiatica* showed 84.83 percent. The plausible reason for high value of AOA in *L. camara* can be attributed to the synergistic effects of TPC, TFC, TAC and essential oils present in the flowers.

3.4.1 Estimation of extract by ABTS$^{\cdot+}$ free radical. The antioxidant capacity of the flavonoids measured by the TEAC method showed that among the four extracts *L. camara* showed highest AOA 413.761 μMT.

3.4.2 Estimation of extract by DPPH free radical. All extracts directly reacted and quenched with DPPH free radical. The extract of *L. camara* showed highest activity 95.72 percent for radical scavenging capacity.

3.4.3 Estimation of extract by FRAP assay. The results were compared with the reference (gallic acid), methanolic extract of *G. asiatica* had the highest reducing power (43.44 GAE/g), whereas *P. betel* was found to have the lowest reducing power.

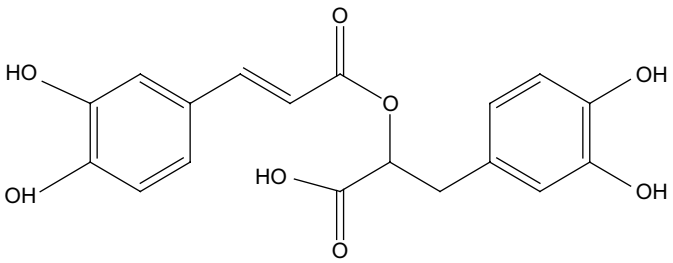

Figure 3.
Structure of rosmarinic acid

Notes: *GA – *G. asiatica*, LC – *L. camara*, OB – *O. basilicum*, PB – *P. betel*

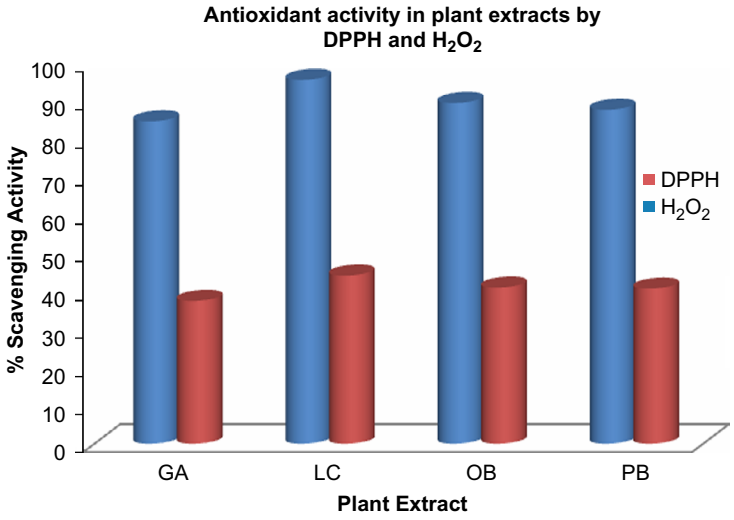

Figure 4.
Antioxidant activity in traditional plants

Plant species	TEAC (μMTE)	FRAP (GAE/g)
G. asiatica (fruit)	269.038	4.14
L. camara (flower)	413.761	24.90
O. basilicum (leaf)	324.522	14.30
P. betel (leaf)	319.994	3.44

Table II.
Antioxidant activity in traditional plants

3.4.4 Estimation of extract by H_2O_2. The methanol extract of the *G. asiatica* showed a strong antiradical effect (37 percent) against peroxyl evaluation of antioxidant. The total phenolic and anti-scavenging activity content present in *G. asiatica* (fruit), *L. camara* (flower), *O. basilicum* (flower) and *P. betel* (leaves) were compared. Anti-scavenging activity of *L. camara* was clearly higher 95.72 percent as compared to other species.

4. Conclusion

This is the first report on the evaluation of antioxidant effects of aforementioned plants and their flavonoids contents. Presence of high phenolic content in each chosen plants clearly indicated the bioactivity to be due to polyphenols and flavonoids. Antioxidant activities were measured with a variety of tests. The high scavenging property of *L. camara* may be due to hydroxyl groups existing in the phenolic compounds that can provide the necessary component as a radical scavenger. *L. camara* and *O. basilicum* showed high AOA. The plausible reason for their high value of AOA could be attributed to the synergistic effects of TPC, TFC, TAC and essential oils present in these plants.

Thus, leaves of *O. basilicum* and flowers of *L. camara* showed an exceptionally high phytochemical profile and can be further developed for medical use and as an alternative herbal source of antioxidant.

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