



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(12)60296-2 ©2012 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

Preliminary phytochemical and antioxidant study of hydroalcoholic extracts from selected genera of Indian Lamiaceae

G. Ramu^{1*}, G. Krishna Mohan², K.N. Jayaveera³, S.P. Dhanapal⁴, G. Senthilkumar⁵¹Department of Phytochemistry, S.A.C College of Pharmacy, Karnataka-571448, India.²Department of Pharmacognosy, J.N.T University, Kukatpally, Andhrapradesh, India.³Dept of Chemistry, J.N.T University College of Engineering, Andhra Pradesh, India⁴Department of Pharmacognosy, JSS College of Pharmacy, Ooty-1, Tamilnadu, India

ARTICLE INFO

Article history:

Received 25 June 2012

Received in revised form 5 July 2012

Accepted 7 August 2012

Available online 28 August 2012

Keywords:

*Salvia officinalis**Plectranthus mollis*

Total flavonoid

Total phenol

Antioxidant capacity

ABSTRACT

Objective: To investigate the antioxidant activity and total phenolics content of the hydroalcoholic extracts of *Salvia officinalis* and *Plectranthus mollis*. **Methods:** The total antioxidant capacity and phenolic contents of hydroalcoholic extracts of the leaves of *Salvia officinalis* and *Plectranthus mollis* were evaluated using standard in-vitro methods. The total phenol and total flavonoid were determined spectrophotometrically. Quercetin and Gallic acid were used for these parameters. The antioxidant activity of the extracts were determined by phosphomolybdenum method. **Results:** Our results clearly demonstrate that both the extracts have antioxidant capacity. The inventions of antioxidant capacity harmonize with total flavonoid and phenol contents. **Conclusions:** A correlation between the antioxidant activity and the total phenolic contents of the extracts indicated that phenolic compounds were the dominant contributors to the antioxidant activity of both the plants. The results of the study show that *Plectranthus mollis* has the most flavonoid and phenol contents and the antioxidant capacity.

1. Introduction

The family Lamiaceae contains several genera, such as sage (*Salvia*), basil (*Ocimum*) and mint (*Mentha*), with a rich diversity of ethnobotanical uses. Another important genus is *Plectranthus*, a large and widespread genus containing about 300 species being found in Tropical America, Asia and Australia [1]. *Plectranthus mollis* (Aiton) Spreng. is native to Asia and infusion of the leaves have been used in the folk medicine to treat inflammations, respiratory infections, vasoconstriction and mental retardations [2]. In India, several ethnomedicinal studies have shown that *Plectranthus mollis* is used as a febrifuge [3]. *Plectranthus mollis* contains fenchone, limonene, piperitenone, β -bisabolene, β -cubebene and

α -humulene [4]. The genus *Salvia* includes about 700 species spread throughout the world. *Salvia officinalis* L is native to Mediterranean region and is commonly known as sage. The infusion and decoction of the leaves have been used as nerve tonic, digestive, antispasmodic and anti-inflammatory in Indian traditional medicine [5]. *Salvia officinalis* contains tannic acid, rosmarinic acid, chlorogenic acid, caffeic acid, steroids, flavones and flavonoid glycosides [6]. Terpenoids, steroids and phenolics are the most common secondary metabolites in *Plectranthus*. The majority of them are highly modified abietanoids, in addition to some phyllocladanes and ent-kaurenes. It seems to be similar to the pattern of diterpenoids of *Salvia* [7], but no clerodane diterpenoids were found in *Plectranthus*. Plant phenolics, in particular phenolic acids, tannins and flavonoids are known to be potent antioxidants and occur in vegetables, fruits, nuts, seeds, roots, leaves and barks [8]. The aim of our study was to investigate the probable antioxidant effects of hydroalcoholic extracts from two genera of Indian Lamiaceae namely *Salvia officinalis*

*Corresponding author: Senior Lecturer, Dept. of Phytochemistry, Sri Adichunchanagiri College of Pharmacy, B.G.Nagar - 571 448, Mandya Dist. Karnataka
Ph: 09972317434
E-mail: ramupharmu@yahoo.co.in

and *Plectranthus mollis* growing wild around Nilgiris region. The second aim of the study was to expose total flavonoid and phenol contents of these plants.

2. Materials and methods

2.1. Chemicals

Chemicals used were as follows; methanol, sodium carbonate, potassium acetate, aluminium chloride, quercetin, gallic acid and Folin–Ciocalteu reagent. All other chemicals used were of analytical grades and purchased from Sigma Life Sciences (Mumbai, India).

2.2. Plant materials collection

Leaves of the plants (*Salvia officinalis* and *Plectranthus mollis*) were collected from Nilgiris region, Tamilnadu, India in summer 2011 and authenticated in Medicinal Plant Collection Unit, Government Arts College, Nilgiris. Voucher specimens (accession no. Pharmacog./1052 and Pharmacog./1053) were deposited for future reference in the Herbarium of Pharmacognosy Department, S.A.C College of Pharmacy, B.G.Nagara, Karnataka, India.

2.3. Extraction procedure

The shade dried leaves were coarsely powdered and extracted with mixture of ethanol : water (7:3 ratios) by a Soxhlet apparatus at 45°C. The obtained extracts were filtered and concentrated in a rotary evaporator at 45°C under reduced pressure, yielding gummy mass for *Salvia officinalis* and *Plectranthus mollis*. These crude extracts were used for further investigation for potential antioxidant properties and total phenolics determination.

2.4. Phytochemical screening

Chemical tests were carried out using standard procedures to identify the constituents as described by Trease and Evans [9] and Harborne [10].

Alkaloids: About 0.2 g of the extracts was warmed with 2 % sulphuric acid for two minutes. It was filtered and few drops of Dragendorff's reagent were added. Orange red precipitate indicates the presence of alkaloids.

Saponins: About 0.2 g of the extracts was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy mass of small bubbles) shows the presence of saponins.

Terpenoids (Salkowski test): 0.2 g of the extracts was mixed with 2 ml of chloroform and 3 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface will form to indicate positive results for the presence of terpenoids.

Steroids (Lieberman–Burchard's test): 2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of sulphuric

acid. The colour change from violet to blue or green in samples indicates the presence of steroids.

Tannins: Small quantity of extracts was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins. 1 ml of extract was added to 2 ml of sodium chloride (2%), filtered and mixed with 5 ml of 1 % gelatin solution. A precipitate indicates the presence of tannin.

Flavonoids: Extracts of about 0.2 g was dissolved in sodium hydroxide and hydrochloric acid was added. A yellow solution that turns colourless, indicates the presence of flavonoids.

2.5. Thin Layer Chromatography

Plant materials were screened to detect flavonoids, cinnamides derivatives, terpenoids, steroids and alkaloids using methods described in Table.2.

The Chromatography was performed by TLC on Silica gel (s.d.fINE–CHEM, Mumbai) developed by different solvent systems: EtOAc–HCOOH–AcOH–H₂O (100:11:11:26 v/v) and EtOAc–HCOOH–AcOH–H₂O (100:0.5:0.5:0.5 v/v).

2.6. Estimation of total phenol content (TPC)

Total phenol content of the extracts was determined colorimetrically using Folin–Ciocalteu method [14]. This test is based on the oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids. The aliquots (400 μ l) of each extract was mixed with 2 ml of Folin–Ciocalteu reagent and 1.6 ml of 4 % sodium carbonate. The mixture was allowed to stand for 2 h with intermittent shaking for reaction. After oxidation the green–blue complex formed was measured at 750 nm (Perkin Elmer, UV–Visible Spectrophotometer). Using gallic acid monohydrate, standard curve was prepared and linearity was obtained in the range of 10–50 μ g/ml. Total phenolics was calculated using the standard curve and the concentrations are expressed as gallic acid equivalent (GAE) in % w/w of the extracts. The calibration equation for gallic acid was $Y = 0.07409X + 0.0587$ ($R^2 = 0.9976$).

2.7. Estimation of total flavonoid content (TFC)

Aluminium chloride colorimetric technique [15] was used for total flavonoids estimation. Flavonoids are capable of forming complexes with metal ions and act as antioxidants. A known volume (1.0 ml) of the extract was mixed with 3ml of methanol, 0.2 ml of 10 % aluminium chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with the help of Perkin Elmer UV–Visible Spectrophotometer. The total flavonoid content was expressed as quercetin equivalent in % w/w of the extracts. The calibration equation for quercetin was $Y = 0.04173 X + 0.014178$ ($R^2 = 0.9995$).

2.8. In-vitro antioxidant assay

2.8.1. Determination of total antioxidant capacity

The total antioxidant capacity was evaluated by the phosphomolybdenum method [16]. The assay is based on the reduction of Mo (VI) to (V) by the antioxidant extract and subsequent formation of a green phosphate Mo (V) complex at acidic pH. An aliquot of 0.3 ml of the extract containing a reducing species in DMSO was combined in an eppendorf tube with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated in water bath at 95 °C for 90 min. Then the absorbance of the solution was measured at 695 nm using spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract was used as the blank. The total antioxidant capacity was expressed as mM equivalent to ascorbic acid per gram dry mass.

3. Results

Phytochemical screening by simple chemical tests and Thin Layer Chromatography showed presence of flavonoids (caffeic acid, quercetin and luteolin), triterpenoids and steroids (β -sitosterol, β -amirin) and cinnamic derivatives (chlorogenic acid) in both the plants (Table 1& 2).

Table.3. shows the total flavonoid and phenol contents, and total antioxidant capacities of the plant extracts. Our results showed that both of these species have antioxidant effect and *Plectranthus mollis* has the most antioxidant activity.

The total phenol content and total flavonoid content showed strong correlation with total antioxidant activity, with the correlation coefficient $R^2= 0.9876$ and $R^2= 0.9881$ for *Salvia officinalis* and *Plectranthus mollis* respectively. This indicates that the antioxidant activity of the extracts from *Salvia officinalis* and *Plectranthus mollis* leaves are due to its phenolic constituents. These results are in accordance with other reports in the literature, which showed positive strong correlation between antioxidant activities and total phenolics [17].

4. Discussion

The most popular traditional plant in our region is *Salvia officinalis*, therefore our aim was to compare antioxidant capacity of the other Lamiaceae member, *Plectranthus mollis* widely available in our region with *Salvia officinalis*.

Plant materials contain many compounds with antioxidant activity. Several plants have been studied as sources of potentially safe natural antioxidants for the food, pharmaceutical and cosmetic industries, various compounds have been isolated many of them being polyphenols [18].

Table 1

Phytochemical screening of hydroalcoholic extracts of leaves of *Salvia officinalis* and *Plectranthus mollis*. (+) – Presence, (–) – Absence.

S.No	Constituents	Tests	Hydroalcoholic extract	
			<i>Salvia officinalis</i>	<i>Plectranthus mollis</i>
1.	Alkaloids	Dragendorffs test	–	–
2.	Saponins	Frothing test	–	–
3.	Terpenoids and Steroids	1. Salkowski test	+	+
		2. Liberman–Burchard’s test	+	+
4.	Tannins	1. Ferric chloride test	+	+
		2. Gelatin test	+	+
5.	Flavonoids test	Sodium hydroxide test	+	+

Table 2

Thin Layer Chromatography of hydroalcoholic extracts of leaves from *Salvia officinalis* and *Plectranthus mollis*. (+) – Presence, (–) – Absence.

Metabolites	TLC solvent	Reagent	<i>Salvia officinalis</i>	<i>Plectranthus mollis</i>
Alkaloids	EtOAc–HCOOH–AcOH–H ₂ O (100:11:11:26 v/v)	Dragendorff	–	–
Saponins	EtOAc–HCOOH–AcOH–H ₂ O (100:11:11:26 v/v)	Anisaldehyde	–	–
Terpenoids and Steroids (β -Sitosterol, β -Amirin)11	EtOAc–HCOOH–AcOH–H ₂ O (100:0.5:0.5:0.5 v/v).	Liberman/Burchard	+	+
Flavonoids (quercetin, caffeic acid, luteolin)12	EtOAc–HCOOH–AcOH–H ₂ O (100:11:11:26 v/v)	2-Aminoethyldiphenylborinate	+	+

Table 3

Total flavonoid and phenol contents, and total antioxidant capacities of the plant extracts.

Extract	Total phenol (%)	Total flavonoid (%)	Total antioxidant capacity (Mm Ascorbic acid/g dry mass)
<i>P. mollis</i>	12.42±2.012	0.721±0.062	3.982±0.265
<i>S. officinalis</i>	8.12±0.625	0.256±0.126	2.165±0.617

Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups [19]. Therefore, the phenolic content of plants may contribute directly to their antioxidant action. Plants belonging to the Lamiaceae family are very rich in polyphenolic compounds. The major phenolic compounds identified in the extracts of *Salvia* and *Plectranthus* are chlorogenic acid, rosmarinic acid, carnosic acid and salvianolic acid [20]. Among these, rosmarinic acid and chlorogenic acid are the major constituents of many *Salvia* and *Plectranthus* species and has strong antioxidant activities because these groups cause phenols to more easily donate hydrogen atoms to activate free radicals to interrupt the chain reaction of antioxidation [21].

The results of the study show that *Plectranthus mollis* has the most flavonoid and phenol contents and antioxidant capacity. In addition, research is continuing to isolate the active compounds responsible for antioxidant activity.

5. Conclusion

The present study confirmed the in-vitro antioxidant potential of *Plectranthus mollis* with results better with those of *Salvia officinalis*. These data further support the view that the leaves of both plants are promising sources of natural antioxidants, and could be seen as potential sources of useful drugs. Nonetheless, further in-vivo studies and purification of the compounds responsible for antioxidant activity are needed.

Conflict of interest statement

We declare that we have no conflict of interests

Acknowledgements

A grant numbered ACCP/R&D/Research Grants/P11/2011–2012 from the Adichunchanagiri Trust funded this study. A special thanks to Prof. Dr. B.Ramesh, Principal, S.A.C College of Pharmacy for his kind support.

References

- [1] Armatu A, Colceru S, Bubueance C, Draghici E, Pirvu L. Evaluation of antioxidant and free scavenging potential of some Lamiaceae species growing in Romania. *Romanian Biotechnological Letters*. 2010; **15** (3): 5274–80.
- [2] Dipak Kuche, Syed Imran, Rupali Shirsat, Dyaneshwar Bhandange. Comparative Phytochemical and Nutritive studies of leaves and stem of three Lamiaceae members. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2011; **2**(3): 1–4.
- [3] Nirmala Devi Kaliappan, Periyavinayagam Kasi Viswanathan. Pharmacognostical studies on *Plectranthus amboinicus* Lour. *International Journal of Green Pharmacy*. 2011; **3**: 182–84.
- [4] Luciana Cristina Borges Fernandes, Carlos Campos Camara, Benito Soto–Blanco. Anticonvulsant activity of extracts of *Plectranthus barbatus* leaves in mice. *Evidence–Based Complementary and Alternative Medicine*. 2011; **12** (1): 1–4.
- [5] Milka Maksimovic Danijela Vidic, Mladen Milos, Marija Edita Solic, Sabaheta Abadzic, Sonja Siljak–Yakovlev. Effect of the environmental conditions of essential oil profile in two Dinavic *Salvia* species: *S.Brachyodon vandas* and *S.officinalis* L. *Biochemical Systematics and Ecology*. 2008; **35**: 473–78.
- [6] Hasan Kirmizibekmez, Hilal Bardakki Aitan, Erika Liktör–Busa, Annamaria Zana, Erden Yesilada, Judit Hohmom, et al. Chemical constituents of *Salvia dichroantha*. *Biochemical Systematics and Ecology*. 2012; **42**: 18–20.
- [7] Van Zyl R.L, Khan F, Edwards T.J, Drewes S.E. Antiplasmodial activities of some abietane diterpenes from the leaves of few *Plectranthus* species. *South African Journal of Science*. 2008; **104**: 1–2.
- [8] Boskou Dimitrios. Sources of natural phenolic antioxidants. *Trends in Food Science and Technology*. 2009; **17**: 505–12.
- [9] Trease GE, Evans WC: Pharmacognosy. 11th edition. Brailliar Tirdel and Macmillan Publishers, London; 1989.
- [10] Harborne JB: Phytochemical Methods. 3rd edition. Chapman and Hall Ltd. London; 1973. p. 135–203.
- [11] Sharma OP, Dawra RK. *J. Chromatogr. Sci*. 1991; **587**: 351–54.
- [12] Markham KR. Techniques of flavonoid identification. London: Academic Press; 1982. p. 175–78.
- [13] Neu R. 1956. *Naturwissenschaften* 43: 82.
- [14] Mahnaz Khanavi, Mannan Hajimahmoodi, Mina Cheraghi–Niroomand, Zahra Kargar, Yousef Ajani, Abbas Hadjiakhoondi, Mohammad R. Oreisi, et al. Comparison of antioxidant activity and total phenolic contents in some *Stachys* species. *African Journal of Biotechnology*. 2009; **8** (6): 1143–47.
- [15] Atanassova M, Georgieva S, Ivancheva K. Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. *Journal of the University of Chemical Technology and Metallurgy*. 2011; **46** (1): 81–88.
- [16] Jaishree V, Shabna V. A Comparative study of invitro antioxidant and DNA damage protection of soxhlet vs microwave assisted extracts of *Michelia champaca* Linn. Flowers. *Indian Journal of Natural Products and Resources*. 2011; **2**(3): 330–34.
- [17] Piluzza G, Bullitta S. Correlations between phenolic content and antioxidant properties in twenty four plant species of traditional ethnoveterinary use in the Mediterranean area. *Pharm Biol*. 2011; **49** (3): 240–47.
- [18] Kanti Bhooshan Pandey, Syed Ibrahim Rizvi. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*. 2009; **2**(5): 270–78.
- [19] Sandhyarani D, Khomdram, Portssangham K. Singh. Polyphenolic compounds and free radical scavenging activity in eight Lamiaceae herbs of Manipur. *Not Sci Biol*. 2011; **3** (2): 108–13.
- [20] Mohammad B. Hossain, Bilyn K. Rai, Nigel P. Brunton, Ana B. Martin–Diana, Catherina Barry–Ryan, et.al. Characterization of Phenolic Composition in Lamaceae species by LC–ESI–MS/MS. *Journal of Agricultural and Food Chemistry*. 2010; **88** (19): 10576–81.
- [21] Avani Patel, Amit Patel, Patel N.M. Determination of polyphenols and free radical scavenging activity of *Tephrosia purpurea* Linn. leaves. *Pharmacognosy Research*. 2010; **2** (3): 152–58.