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Ethnobotanical, Phytochemical and *invitro* antioxidant activity of medicinal plant *Pimenta dioica* (l.) Merr. (Myrtaceae) from Attappadi, Palakkad district, Kerala

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An ethnobotanical study was undertaken to collect information about the use of medicinal plants in Attappadi hills, Palakkad district, Kerala during December 2012 to February 2013. A total of 51 species of ethanomedicinal plants belonging to 30 families were reported with the help of standardized questionnaires. This survey is mostly useful for rural area of local people. Total phenol and DPPH activity was carried out for *Pimenta dioica*. The aqueous stem extract of *P.dioica* showed higher total phenolic content and acetone extract exhibited the more free radical scavenging activity. These findings show that the plant provide substantial antioxidant activity.

Key words: Ethnobotanical study, Attappadi hills, *Pimenta dioica*, Total phenol and DPPH activity

Introduction

The use of plants as medicine was practiced by our ancestors, a process which must have started by trial and error. In India, traditional healers are reported to use 2500 species, in which 100 species of plants are found to serve as regular source of medicine (Ayyanar and Ignachimuthu, 2005). In recent years, use of ethnobotanical information in medicinal plant research has gained considerable attention in segments of the scientific community (Heinrich, 2000). Recently, plants were important sources for the discovery of novel pharmacologically active compounds, with many blockbuster drugs being derived directly or indirectly from plants (Newman and Cragg, 2007; Li, 2010). The tribal people and ethnic races throughout the world have developed their own culture, customs, religious-rites, folk songs, medicinal practices etc. Numerous wild and cultivated plants play a key role among tribal cultures and this relationship has been continuing from one generation to another. Thus a study has been conducted in Agali, Mukkali and Anavay villages of Attapadi hills, Palakkad district, Kerala which has pre-dominance of such traditional healers.

The plant *Pimenta dioica* (L.) Merr. Syn. *Eugenia pimenta* D.C., *Myrtus pimento* L., *Pimenta officinalis* Lindl., *Pimenta vulgaris* Lindl. belongs to the family Myrtaceae, is a tree native to tropical ever green rain forest of central America region. The essential oil of *Pimenta dioica* leaves and fruits are utilized in food industries-viz, meat and canning industries, in perfumery compositions and cosmetic products. The therapeutic properties of the essential allspice oils anesthetic, analgesic, antimicrobial, antioxidant, antiseptic, acarcidal, carminative, muscle relaxant, rubifacient, stimulant and tonic. Pimento oil can be helpful for the digestive system, for cramp, flatulence, indigestion and nausea. The aim of

the present study is to measure the antioxidant properties of ethanomedicinal plant *Pimenta dioica* which was used by the peoples of Attappadi hills of Palakkad.

Materials and method

Study area

Attappadi is one of the prominent tribal regions of Kerala with abundant vegetation and extensive forest. The study areas Agali, Mukkali and Anavay are small villages in Attappadi block of Palakkad district, Kerala. The hills are bordered to the East by Coimbatore district in Tamilnadu, on the North by the Nilgiris, south by Mannarkkad taluk and west by Palakkad and Malappuram districts at 10º55′ - 11º15′N latitude and 76º21′ - 76º48′E longitude. The mean sea level of study area ranging from 450 to 2300m msl.

The area is tropical in climate and vegetation characterized by "Moist evergreen forest of the slopes and at low elevation" and shrub savanna called sholas. The study area receives rain fall during the south west monsoon (June-September), it constitutes about 70% of the annual rainfall. Average annual rainfall is 5440mm.

Ethnobotanical study

The ethnobotanical surveys were carried out during the period of December 2012 to February 2013 for documentation of information and collection of plant material. A detailed survey was carried out in the villages namely Agali, Mukkali and Annavay in Attappadi hills. This study is based on an independent field investigation among the Mudhuga, Irula and Kurumba employing mainly methods of participants, observation and unstructured interviews. The field visit was conducted many times to the study area.

Ethnobotanical data was collected according to the methodology suggested by Jain (1964), through questionnaire, interviews and discussions among tribal practitioners in their local language. Very famous traditional healers and other versatility people of each area were interviewed to document detailed information on local names, folklore plants, plant parts used and all other kinds of details offered by the informants. Local names, useful plant parts, methods of preparation and dosage were recorded. Identification and nomenclature of the collected plants were done based on the Flora of Presidency of Madras (Gamble, 1936) and confirmed in the herbarium of Department of Botany, Kongunadu Arts and Science College, Coimbatore. Data are tabulated with plant name along with family, local name, parts used and utility.

Plant Materials

Different parts of stem of *Pimenta dioica* were collected during November 2012 from Attappadi, Palakkad District, Kerala, India. With the help of local flora, a voucher specimen was retained in the Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu for further reference.

Extraction of Plant Material

Various organic solvents (acetone, ethanol and aqueous) were used for the extraction of bioactive compounds. The shade dried powdered plant material was subjected to aqueous, acetone and ethanolic extraction by cold extraction methods. The extracts obtained were completely evaporated using rotary vacuum evaporator. The concentrated extracts were used for phytochemical and antioxidant activity.

Qualitative phytochemical analysis

The phytochemical screening of the samples such as alkaloids, steroids and saponins were carried out as described by Sofowora (1993) and Harbone (1973).

Total Phenolic Content

The total phenolic content of the extract was determined using the method of Macdonald *et al*. (2001) with slight modifications. Absorbance values were measured at 765 nm and the standard curve was drawn after an incubation of 40 minutes in dark to determine the total phenolic content. All determinations were carried out in triplicate. The total phenolic content in the extract were presented as mg Gallic Acid Equivalents (GAE)/ g extract.

DPPH• scavenging activity

DPPH (1,1-diphenyl-2-picryl hydrazine) free radical-scavenging capabilities of methanolic extracts were evaluated by the method of Blois (1958). Briefly, different concentrations (50, 100,150,200 and 250 mg/ml) of the extracts were pipetted out to the test tubes. 100µL of 0.2 mM alcoholic DPPH solution was added to the samples. These samples were vortexed, and incubated in dark at room temperature for 30 min. The absorbance was measured at 517 nm against blank samples. Decreased absorbance of the sample indicates DPPH• free radical scavenging capability (Gulcin, 2004a; 2004b).

RESULTS AND DISCUSSION

The present communication documented 51 plant species belonging to 30 families that are being traditionally used in the area. Maximum number of medicinal plant species belongs to family Rutaceae (5 species) followed by Asclepiadaceae, verbinaceae, Lamiaceae, Euphorbiaceae (3 species), Menispermaceae, Myrtaceae, Papilionaceae, Cucurbitaceae, Apocynaceae, Scrophulariaceae, Acanthaceae, Amaranthaceae, Zingiberaceae, Amaryllidaceae and Asteraceae (2 species), and others with one species (Table 3). Based upon plant habit, climbers, herbs, shrubs and trees have the percentage share of 14, 37, 31 and 18% respectively (Fig. 1). In the present study, a brief account on ethnomedicinal uses of documented plant species has been verified by tribes, knowledgeable persons and experienced informant of the area, even then further exploration on pharmaceuticals, therapeutic as well as safety features like toxicity studies are very much required for human benefit and sustaining the knowledge of tribal communities.

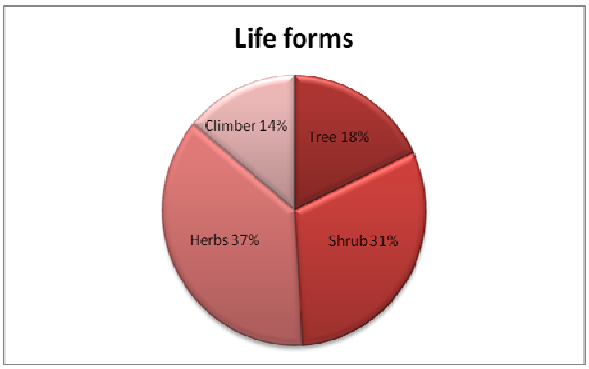

Fig. 1: Habit pattern of different plant species recorded in study area

Balasubramanian and Narenndra Prasad (1996) enumerated the medicinal plants among the Irula of Attappady. This area was also observed by Binu *et al.* (2003) in which they enumerated 40 medicinal plant species belonging to 23 families used as medicine by the Irula of Palakkad district, Kerala.

The qualitative analysis of secondary metabolites using aqueous, acetone and ethanolic extract of crude stem of *Pimenta dioica* confirmed the presence of alkaloid,

flavonoid, glycosides, phenols, steroids, phytosteroids, terepinoids, tannins, cardiac glycosides, fats, gum and mucilage (Table 1). The phenolic content of *Pimenta dioica* aqueous stem extract is higher than that of acetone and ethanol extracts. Similarly, free radical scavenging potential of *Pimenta dioica*, acetone stem extract is much higher than that of ethanolic and aqueous extracts (Table 2).

Table 1: Qualitative phytochemical analysis of *Pimenta dioicastem* extract

Phytochemical test	Aqueous	Acetone	Ethanol
Carbohydrate	+	++	++
Tannin	+	++	++
Saponin	++	+	-
Flavonoid	++	++	++
Alkaloid	+	+	+
Quinones	+	++	++
Glycosides	-	+	+
Cardiac glycosides	-	++	++
Terpinoid	+	++	++
Phenols	++	++	+
Coumarins	++	++	++
Acids	-	-	-
Proteins	+	+	+
Steroids	++	++	++
Phytosteroids	++	++	++
Phlobatannin	-	-	-
Fat	+	+	+
Gum and Musilage	+	+	+
Phytosterol	-	-	-

Table 2: Total phenolics and DPPH activity of *Pimenta dioicastem* in various extracts.

Plant part used	Total phenolics(GAE/g of sample)	DPPH (IC_{50} value)
SA	6.36±8.9	205 ±6.36
SE	6.95±9.4	250±5.32
SW	16.31±0.5	400±3.05

Table 3 -Inventory of Herbal Medicines in Attappadi Hills of Palakkad District, Kerala.

S.No	Botanical name	Family	Local Name	Habit	Parts used	Aliments Treated
1.	*Cyclea peltata* (Lam.)	Menispermaceae	Pada thali	Climber	Leaves	Jaundice, stomach pain, poisoning and asthma
2.	*Pachygone oveta* (Poir.)	Menispermaceae	Pothidag	Climber	Leaves	Gas trouble
3.	*Helicteres isora* L.	Sterculaceae	Edampuri	Shrub	Root, Bark, Fruit	Diabetes, verbal pain, ulcer and menstrual pain
4.	*Aegle marmalos* (L.)	Rutaceae	Koovalam	Tree	Leaves, fruit, seed	Constipation, chronic dysentery, dyspepsia
5.	*Citrus limon* Linn.	Rutaceae	Ellumiche	Tree	Leaves, fruit	Indigestion

6.	*Glycosmis pentaphylla* (Retz.) DC.	Rutaceae	Malakuluki	Shrub	Leaves, roots	Dysentery and stomach pain
7.	*Muraya konigi* Spreng.	Rutaceae	Kariveppila	Tree	Leaves, fruit	Stomach pain
8.	*Todalia asiatica* (L.)	Rutaceae	Erigotha	Climber	Root	Gas trouble, ulcer, antipyretic digestion
9.	*Azadiracta indica* Adr.	Meliaceae	Arya vepu	Tree	Whole plant	Gas trouble in cattle and intestinal worms
10.	*Cissus quadrangularis* L.	Vitaceae	Changalam paranda	Shrub	Stem	Stomach ache and dysentery
11.	Cardiospermum helicacabum L.	Sapindaceae	Valliulinga	Climber	Leaves, roots, seeds	Stomach upset
12.	*Mangifera indica* Linn.	Anarcardiaceae	Mavu	Tree	Leaves	Dysentery
13.	*Mimosa pudica* L.	Mimosaceae	Thottavadi	Shrub	Leaves, root, seed	Kidney stone and dysentery
14.	*Indigofera spicata* Forsk.	Papilionaceae	Pulincheera	Herb	Roots	Stomach pain
15.	*Phyllodum pulchellum* Desv.	Papilionaceae	Kolatta	Shrub	Roots	Acidity and malaria
16.	*Psidum guajava* Linn.	Myrtaceae	Sarvasuganthi	Tree	Leaves, stem, fruit	Cramp, flatulence and indigestion
17.	*Coccinia indica* L.	Cucurbitaceae	Kovachedi	Climber	Leaves	Dysentery
18.	*Momordia charantia* Linn.	Cucurbitaceae	Kaypakka	Herb	Leaves, fruit	Diabetes and intestinal worm
19.	*Centella asiatica* (L.)	Apiaceae	Kudangal	Herb	Whole plant	Dysentery, memory tonic
20.	*Ageratum conyzoid* Linn.	Asteraceae	Communist appa	Herb	Leaves	Ulcer
21.	*Tridax procumbens* Linn.	Asteraceae	Vettupakku	Herb	Leaves	Stomach pain
22.	*Plumbago zeylanica* Sp.	Plumbaginaceae	Vella koduvelli	Shrub	Leaves, root	Stomach pain
23.	*Alstonia sholaris* R.	Apocynaceae	Azhilam pala	Tree	Leaves	Dysentery, fever
24.	*Rauvolfia erpentine* Benth.	Apocynaceae	Sarpaganthi	Shrub	Roots	Dysentery, diarrhea
25.	*Pergularia daemia* (Forssk.)	Asclepiadaceae	Veliparuthi	Climber	Whole plant	Gas touble and breathing trouble in cattle
26.	*Hemidesmus indicus* (L.)	Asclepiadaceae	Nannari	Shrub	Roots	Dysentery
27.	*Solanum melongina* Linn.	Solanaceae	Kathirika	Shrub	Fruit	Stomach pain
28.	*Bacopa monnieri* (L.) Pennel	Scrophulariaceae	Brahmi	Herb	Stem	Reduce fat and cure stomach ulcer
29.	*Scoparia dulcis* L.	Scrophulariaceae	Kalluruki	Herb	Whole plant	Kidney stone
30.	*Adhatoda vasica* Nees in Wallich.	Acanthaceae	Adalodakam	Shrub	Leaves, root	Bronchitis and acidity
31.	*Elephantopus scaber* Linn.	Acanthaceae	Anachuvadi	Herb	Whole plant	Dysentery
32.	*Callicarpa tomentosa* (L.) Murr.	Verbinaceae	Karumpeetha maran	Tree	Leaves	Diarrhea
33.	*Clerodendrum serratum* (L.) Moon	Verbinaceae	Seruthekku	Shrum	Roots	Stomach ache

34.	*Lantana indica* Roxb.	Verbinaceae	Vettum paral	Shrub	Leaves	Stomach ache and control vomiting
35.	*Coleus barbatus* Benth.	Lamiaceae	Panikoorka	Herb	Leaves	Diarrhea
36.	*Leucas aspera* (Willd).	Lamiaceae	Thumba	Herb	Whole	Stomach pain
37.	*Ocimum sanctum* Sims in Curtis	Lamiaceae	Kattuthulasi	Herb	Whole plant	Vomiting
38.	*Curcuma zedoria* Christum.	Zingiberaceae	Kachola kizhangu	Shrub	Tuber	Indigestion
39.	*Zingiber officinale* Rosc.	Zingiberaceae	Inji	Shrub	Tuber	Indigestion
40.	*Boerhhavia diffusa* L.	Nyctaginaceae	Thazhuthama	Herb	Whole plant	Stomach ulcer
41.	*Achyranthus aspera* L.	Amaranthaceae	Nayuruvi	Sub-shrub	Whole plant	Abortion and diuretic
42.	*Aerva lanata* Juss.	Amaranthaceae	Cherula	Herb	Whole plant	Abdominal disorder
43.	*Aristolochia indica* Linn.	Aristolochiaceae	Mudhulaikodi	Climber	Leaves	Stomach pain
44.	*Piper nigrum* Linn.	Piperaceae	Melagu	Climber	Seeds	Stomach pain
45.	*Euphorbia hirta* L.	Euphorbiaceae	Nilappana	Herb	Whole plant	Cough and dysentery
46.	*Ricinu communis* L.	Euphorbiaceae	Kattamanaku	Shrub	Seed oil, roots, leaves	Dysentery and jaundice
47.	*Tragia involucrate* Sp.	Euphorbiaceae	Kodithuva	Climber	Whole plant	Indigestion
48.	*Allium cepa* Linn.	Amarilidaceae	Vengayam	Herb	Bulbous	Indigestion
49.	*Allium sativum* Linn.	Amarilidaceae	Vellavengayam	Herb	Bulbous	Indigestion
50.	*Aloe vera* (L.)Burm.	Liliaceae	Chothukathala	Herb	Whole plant	Jaundice, dysentery and menstrual problem
51.	*Kyllimga melanosperma* Nees in Wight.	Poaceae	Muthanga	Herb	Bulbous	Dysentery and white discharging

Conclusion

The tribal people use several indigenous plants in their daily life style as well as to cure some important diseases in the day to day treatments. The native inhabitants were well versed with the utilization of plants of their surroundings. So this study will be helpful for research and pharmaceutical industries to find out the other uses of plant which will be helpful to the modern health care system of India.

The plant extractive study could be an answer to the people seeking for better therapeutic agents from natural source it is believed to be more efficient with little or no side effects when compared to the commonly used synthetic chemotherapeutic agents. The present study verified the traditional use of *Pimenta dioica* for human ailments such as rich source of phytochemicals and antioxidants. Thus, this plant can be utilized by an alternative source of useful drug.

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