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Determining the antioxidant activity of certain medicinal plants of Attapady, (Palakkad), India using DPPH assay

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ABSTRACT

Cellular damage or oxidative injury arising from free radicals or reactive oxygen species (ROS) now appears the fundamental mechanism underlying a number of human neurodegenerative disorders, diabetes, inflammation, viral infections, autoimmune pathologies and digestive system disorders. Free radicals are generated through normal metabolism of drugs, environmental chemicals and other xenobiotics as well as endogenous chemicals, especially stress hormones (adrenalin and noradrenalin). Accumulated evidence suggests that ROS can be scavenged through chemoprevention utilizing natural antioxidant compounds present in foods and medicinal plants. India is blessed with enormous biodiversity resources, but plagued with several diseases, including those with ROS as the etiological factor. In this study the antioxidant activity and radical scavenging activity of methanolic extracts of selected plant materials, traditionally used by the tribes of Attapady regions as folk remedies was evaluated against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. *Cassia occidentalis*, *Clitoria ternatea*, *Trianthema decandra*, *Capparis zeylanica*, *Anisomeles malabarica* and *Plumbago zeylanica* exhibited strong antioxidant activity as compared to other plants. *Trianthema decandra* showed the highest antioxidant activity. The present study reveals that these plants are of therapeutic potential due to their high free-radical scavenging activity.

Introduction

Free radicals (super oxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and proxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA [1, 2]. It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others [3-8]. The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants, both exogenous or endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders [9-11].Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders. For instance in carcinogenesis, reactive oxygen species are responsible for initiating the multistage carcinogenesis process. Epidemiological and *in vitro* studies on medicinal plants and vegetables strongly supported this idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems [12-16]. Recently, there has been growing interest in natural antioxidants of plant origin because they have greater application in the food industry for increasing the stability and shelf life of food products. Moreover, they also find use as nutraceuticals and phytoceuticals as they have significant impact on the status of human health and disease prevention 17]. The Indian subcontinent represents one of the greatest emporia of ethno biological wealth and Western Gnats represents the second hot spot in India [18, 19]. Palakkad is a central district in Kerala that lies at the foot of the Western Ghats. In Kerala, many living groups of tribals, still more or less unaffected by urbanization continue to use various plants for food, drugs, customs, game, religious purposes etc. Attapady is inhabited by tribals like *Irulas, Kurumbas* and *Murugas*. Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary healthcare system of resource poor communities and the local therapy is the only means of medical treatment for such communities. The traditional herbal knowledge is passed from generation to generation in the verbal form by traditional medicinal man or '*vaidyar*'. However, since cultural systems are dynamic [20], the skills are fragile and easily forgettable as most of the indigenous knowledge transfer in the country is based on oral transmission [21]. To our knowledge, there are no data regarding the traditional medicinal plant knowledge and use by the local communities in Palakkad District. In view of the above, the current study was carried out to evaluate antioxidant activity of certain medicinal plants of Palakkad district of Kerala, India.

Materials and Methods

Plant Material and Extraction Procedure: The plants were collected from around the Attapady area (Palakkad district) in Kerala and were identified with the help of relevant literature and authenticated at the Botanical Survey of India, Coimbatore and the voucher specimen of the collected plants were deposited at the departmental herbarium. The Baseline information on the medicinal utility of these plants was also collected by carrying out literature survey (Table 1). Different plant parts were shade-dried at room temperature and ground in a mortar. 50g of the powdered plant material was extracted with 500 ml of methanol for 3 consecutive days. The coloured solution obtained from each of the plant material was concentrated by Rotary evaporator [22]. The concentrate (extract) obtained was preserved at 20 ^{0}C for further experimentation.

Phytochemical analysis: All the plant extracts were qualitatively tested for the presence of chemical constituents (Phenols, Tannins, Alkaloids, Flavonoids and Saponins) (Table 2).

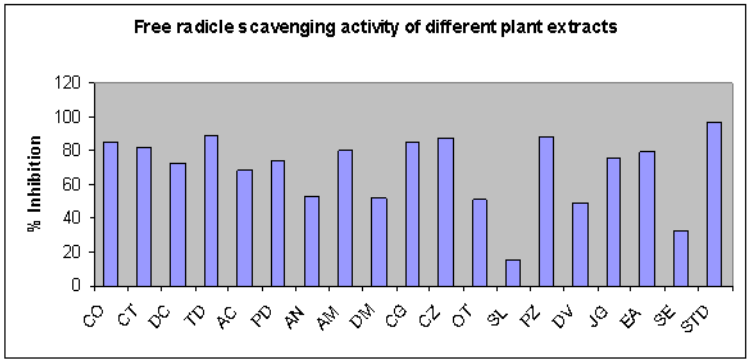
Free radical scavenging activity: The antioxidant activities were determined using DPPH, (Sigma-Aldrich, Germany; M.W. 394.32) as a free radical. 1 mg/ml solution of plant extract in methanol was prepared. 6 x 10-5 mol/L DPPH in methanol was prepared. 0.1 ml of plant extract was added to 3.9 ml of DPPH solution. The decrease in absorbance at 515nm was recorded at 1 min interval upto 15 min or until the reaction reached a plateau. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control [23]. Ascorbic acid (Merck; M.W. 176.13) was used as standard. The experiment was carried out in triplicate. Free radical scavenging activity was calculated by the following formula:

% DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance Of control)] x 100

Results

In this study 18 medicinal plants, traditionally used in Palakkad, India for various disorders were studied for their antioxidant activity against DPPH free radical scavenging assay. *Trianthema decandra* was the most active antioxidant .The baseline information (botanical name, family, local name, medicinal utility and part used) of the selected plants has been presented in Table 1.Phytochemical constituents of the plants under study have been depicted in Table 2.

Scavenging activity of different plant extracts on DPPH radical has been shown in Fig. 1. As evident from the Fig. 1, there was noticeable variability in the antioxidant activity of plant extracts. Out of Eighteen plants screened, *Cassia occidentalis, Clitoria ternatea, Capparis zeylanica, Anisomeles malabarica* and Plumbago *zeylanica* were found to be good radical scavengers with the percent inhibition of 84.23±0.004, 82.87±0.246, r87.12±0.004, 80.12±0.008 and 88.45±0.001 respectively whereas the per cent inhibition for Ascorbic acid was 96.540 ± 0.652 which is used as a standard. Tannins play important role in promoting wound healing. This could be the reason why the bark paste of *A. nilotica* is applied on wounded portion of the body to give relief from pain (Table 1). In addition, the presence of flavonoids and tannins (Table 2) suggests the reason why the root bark of *P. zeylanica* is used for the treatment of diarrhoea (Table 1). Flavonoids can inhibit the development of fluids that result in diarrhoea by targeting the intestinal cystic fibrosis transmembrane conductance regulator Cl– transport inhibiting cAMP-stimulated Cl– secretion in the intestine [24]. Different antioxidant and radical scavenging activity may partly be due to wide variety of antioxidant constituents such as phenolics, ascorbate and carotenoids. Also two types of antioxidants, inhibitors of free radicals which initiate oxidation and inhibitors of free radical chain propagation reactions, are known. Different mechanism of action and kinetics of the inhibitory effect of these antioxidants using different procedures resulted in the discrepancy of these findings ([25-27]. Owing to the complexity of the antioxidant materials and their mechanism of actions, it is obvious that no single testing method is capable of providing a comprehensive picture of the antioxidant profile of a studied sample and a combination of different methods is necessary. Despite such limitations, DPPH free radical scavenging assay can be helpful for primary screening and finding of novel antioxidants [28].



*CO: *Cassia occidentalis*; CT: *Clitoria ternatea*; DC: *Dichrostachys cinerea*; TD: *Trianthema decandra*; AC: *Acacia catechu*; PD: *Pergularia daemia*; AN: *Acacia Nilotica*; AM: *Anisomeles malabarica*; DM: *Datura Metel*; CG: *Capparis grandiflora*; CZ: *Capparis zeylanica*; OT: *Orthosiphon thymiflorus*; SL: *Spermacoce latifolia*; PZ: *Plumbago zeylanica*; DV: *Dodonaea viscosa*; JG: *Justicia glauca*; EA: *Evolvulus alsinoides*; SE:: *Secamone emetica*; STD: Standard (Ascorbic acid)

Fig 1: DPPH radical scavenging activity of different plant extracts

Table 1: Ethno botanical information of different plants

Scientific name	Family name	Local name	Common name	Parts used	Modes of uses	Voucher specimen No.
Cassia occidentalis	Caesalpiniaceae	Ponnavarai, Kasonda	Negro coffee	Whole plant	Decoction (one glass two to three times daily) in general infections.	BSI/SRC /Tech-945
Clitoria ternatea.	Fabaceae	Aparajit	butterfly pea	Root, stem, flowers	Root juice; externally applied for headache and swelling.	BSI/SRC /Tech-933
Dichrostachys cinerea	Fabaceae	Vedathalan	bell mimosa	Whole plant	Paste with curd taken once a day in fever and pain.	BSI/SRC /Tech-941
Trianthema decandra	Aizoaceae	Gadabani	vellai sharuni	Whole plant	Leaf juice is dropped into the nostrils to relieve headache	BSI/SRC /Tech-937
Acacia catechu	Leguminosae	Katha	Black catechu	Bark	The water decoction is consumed as health drink	BSI/SRC /Tech-944
Pergularia daemia	Asclepiadaceae	Utranajutuka	Not found	Whole plant	Decoction of leaf as an inhalation in fever and anaemia.	BSI/SRC /Tech-935
Acacia Nilotica	Mimosaceae	black babul / babul tree	Egyptian Mimosa	Whole plant	Bark infusion and seed powder in diarrhoea.	BSI/SRC /Tech-942
Anisomeles malabarica	Labiatae	Aruvaachadachi	Malabar catmint	Leaves	Essential oil used externally in rheumatism	BSI/SRC /Tech-940
Datura metel	Solanaceae	Datura	Thorn-apple, Devil trumpet	Leaves	Asthma, depression, motion sickness and analgesic, hallucinations.	BSI/SRC /Tech-938
Capparis grandiflora	Capparidaceae	Kevisi	Caper plant	Leaves, root	Decoction given orally(one glass 2-3 times daily) in jaundice.	BSI/SRC /Tech-565
Capparis zeylanica	Capparidaceae	Godanthi	Indian caper	Leaves, root	The watery extract used in cataract	BSI/SRC /Tech-566
orthosiphon thymiflorus	Lamiaceae	pratanika	Not found	Whole plant	External application(two to four times daily)	BSI/SRC /Tech-

						936
Spermacoce latiflolia	Rubiaceae	Shankham	Button weed	Leaves	External application(two to four times daily) in headache.	BSI/SRC /Tech-948
Plumbago zeylanica	Plumbaginaceae	Agnimaala	White lead wort	Root, bark, seeds	Extract of root bark used orally in diarrhoea	BSI/SRC /Tech-939
Dodonaea viscosa	Sapindaceae	aliar and vilayati mehandi	Sticky hop bush	Whole plant	Decoction given orally(two teaspoons daily) to heal wounds.	BSI/SRC /Tech-932
Justica glauca	Acanthaceae	------	Glaucous Justica,Water willow	Leaves, stems	stems used as fumigants	BSI/SRC /Tech-934
Evolvulus alsinoides	Convolvulaceae.	Sankhapuspi	Not found	Whole plant	neurodegenerative diseases, asthma and amnesia,adaptogenic,,antibacterial ,anthelmintic antioxidant ,immunomodulator.	BSI/SRC /Tech-947
Secamone emetica.	Asclepiadaceae	Angaravalli	Not found	Fruits, leaves	Juice with milk taken orally	BSI/SRC /Tech-946

Table 2: Chemical Groups identified in the plant extracts

S.No	Plant extracts	Part used	Phenols	Tannins	Alkaloids	Flavonoids	Saponins
1	*Cassia occidentalis*	Whole plant	+	+	+	+	+
2	*Clitoria ternatea*	Root	+	+	+	+	+
3	*Dichrostachys cinerea*	Whole plant	+	+	+	+	-
4	*Trianthema decandra*	Whole plant	+	+	+	+	+
5	*Acacia catechu*	Bark	+	+	+	+	-
6	*Pergularia daemia*	Whole plant	+	-	+	+	+
7	*Acacia Nilotica*	Whole plant	+	+	+	+	+
8	*Anisomeles malabarica*	Leaves	+	+	+	+	+
9	*Datura metel*	Leaves	+	+	+	+	+
10	*Capparis grandiflora*	Leaves	+	-	+	+	-
11	*Capparis zeylanica*	Leaves	+	+	+	+	-
12	*Orthosiphon thymiflorus*	Whole plant	+	-	-	+	-
13	*Spermacoce latiflolia*	Leaves		+	-	+	-
14	*Plumbago zeylanica*	Root		+	+	+	+
15	*Dodonaea viscosa*	Whole plant		+	+	+	+
16	*Justica glauca*	Leaves		+	+	+	-
17	*Evolvulus alsinoides*	Whole plant		+	+	+	+
18	*Secamone emetica*	Leaves		-	+	+	-

Conclusion

In the present study, the antioxidant activity of eighteen traditionally used medicinal plants grown around the Attapady regions (Palakkad district), Kerala, India was evaluated. The results of the present study suggest that tested plant materials have moderate to potent antioxidant activity and/or free radical scavenging activity. However, we do not know what components in the plant extracts show these activities. More detailed studies on chemical composition of the plant extracts, as well as other *in vivo* assays are essential to characterize them as biological antioxidants which are beyond the scope of this study. It should also be kept in mind that antioxidant activity measured by *in vitro* methods may not reflect *in vivo* effects of antioxidants [29]. Many other factors such as absorption/metabolism are also important. The findings of this study support this view that some medicinal plants are promising sources of potential antioxidant and may be efficient as preventive agents in some diseases. The providing data can just enrich the existing comprehensive data of antioxidant activity of plant materials.

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