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Research Article

DPPH –ANTIOXIDANT ACTIVITY SCREENING OF ELEVEN NATURALLY GROWING PLANTS OF GUJARAT, INDIA

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ABSTRACT
The plants synthesize numerous potent antioxidants to overcome damages caused by active oxygen. Plants contain certain antioxidant phyto-chemicals, which are capable of exerting antioxidant effect by quenching various free radicals.

In the present investigation, effort was made to test the antioxidant capacity of selected naturally and commonly growing plants of Gujarat region. Plant extracts were prepared in various solvents like water, methanol, chloroform and petroleum ether for comparative analysis and to identify the most effective extract fraction in the % DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical scavenging assay. On the whole, plant extracts of *Polyalthia longifolia*, *Pongamia pinnata*, *Petunia violacea*, *Lantana camara*, *Cassia tora*, *Ocimum canum*, *Citrus limon* and *Salvadora persica* have demonstrated presence of an excellent antioxidant activity in the present study.

Key Words: *DPPH, Antioxidant, Free radicals, Phyto-chemicals, Extracts.*

INTRODUCTION

Antioxidants are free radical scavengers. In a natural system, they interrupt the harmful free radicals and form a stable radical (Kitazurua *et al,* 2004; Aluyor and Ori-Jesu, 2008; Mandal *et al,* 2009).

Bio-chemically, antioxidant is a molecule capable of slowing or preventing the oxidation process initiated by reactive free radicals and chelating metals by acting as a radical scavenger, singlet or triplet oxygen quenchers, peroxide decomposers, enzyme inhibitors and synergists (Shyamala *et al,* 2003; Politeo *et al,* 2006; Wu *et al,* 2009).

Bioactive phyto-chemicals like phenolics, flavonoids, anthocyanins and carotenoids present in essential oils and extracts of several spices and herbs are well known to exert antioxidant and antimicrobial activities (Patel and Jasrai, 2009).

The antioxidant role of secondary metabolites with medicinal effects have an important role in long term health, like they reduce the risk of chronic and degenerative diseases and prevent cellular damage in the body (Aquil *et al,* 2006; Patel and Jasrai, 2012). Many researches are going on to find newer and newer sources of natural antioxidants with broad-spectrum actions.

As majority of the rich diversity of Indian medicinal and other plants are yet to be scientifically evaluated for such properties, there is an attempt made here to find the potential antioxidant plant sources. In the present investigation, eleven naturally growing plants (fig. 1) were screened for the presence of antioxidant activity using DPPH RSA (radical scavenging assay). DPPH is 1, 1-diphenyl-2-picryl hydrazyl radical and is commonly used antioxidant model system to investigate the scavenging activities of natural compounds and crude extracts of plants (Nikhat *et al,* 2009).

Figure 1: Plants used for antioxidant screening by DPPH-RSA

Table 1: Phyto-chemical constituents and Medicinal importance of plant parts used

Ailanthus excelsa
Phyto-chemical constituents: Flavones like apigenin, apigeninglucoside, luteolin, luteolinglucoside and flavonols like kaempferol and quercetin.
Uses: Leaves possess insecticidal, febrifuge, antileukemic, antibacterial, antifungal, antifertility activities and CNS stimulant. Also used to cure bronchitis, asthma, worm infestations, wounds, skin eruption, gynecological diseases, diarrhea and dysentery.
Source: Crespi-perellino *et al*, 1986; Heisey, 1990; Said *et al*, 2010
Calotropis procera
Phyto-chemical constituents: Calotropin, calotoxin, uscharin, usecharidine, calotropaginin, calotropain, α-caotropin, giigentin, gigenteol, β-sitosterol.
Uses: Leaves are bactericidal, antioxidant, emeto-cathartic, purgative, digitalic properties, laxative, local anesthetic, weak antipyretic, diuretic, stomach tonic, anti-inflammatory, analgesic, antimalarial, anti-diarrhoetics, antiulcer, anticoagulent and anticancer. Cure intermittent fever, cold, toothaches, arthralgia, swellings, indigestion, liver disorders, skin disorders, whooping cough, asthma, conjunctiva, ophthalmic disorders, colic, jaundice, anorexia, rheumatism, sore gums and ulcers treatment.
Source: Kawo *et al*, 2009; Dwivedi *et al*, 2010; Yadav *et al*, 2010
Cassia tora
Phyto-chemical constituents: Emodin, tricontan-1-ol, stigmasterol, β-sitosteral-β-D-glucoside, freindlen, uridine, quercitrin, isoquercitrin, anthraquinones, chrysophanol, obtusifolin, obtusin.
Uses: Leaves are acrid, liver and cardio tonic, expectorant, antibacterial, antifungal, carminative, stomachic, emollient, laxative, antiperiodic, anthelmintic, anodyne, anticholesterolemic, anti-platelet aggregation, anti-inflammatory, antiestrogenic, hypolipidemic, antimutagenic, antispasmodic and

ophthalmic. Also used to cure leprosy, ringworm, itching, flatulence, colic, dyspepsia, cough and bronchitis.
Source: Sharma *et al*, 2010

Citrus limon
Phyto-chemical constituents: Limonene, terpinene, pinene, sabinene, myrcene, citral, linalool, geraniol, octanol, nonanol, citronellal, bergamotene, cadinene, bisabolene, camphene and phellandrene.
Uses: Leaves are antioxidant and immunomodulatory.
Source: Coventry and Allan, 2001; Seenivasan *et al*, 2006

Clerodendrum inerme
Phyto-chemical constituents: Inerminosides, 3-iridoid glycosides, β-sitosterol, sterols and megastigmane glycosides sammangaosides.
Uses: Leaves can cure skin ailments, fever, cough, skin rashes, boils, topical burns, elephantiasis, asthma, oedema, tetanus, atopic rhinitis, epilepsy, umbilical cord infections, rheumatism and aid cleaning of uterus.
Source: Rajasekaran and Ponnusamy, 2006

Lantana camara
Phyto-chemical constituents: Lancamarone, lantanone, lantanoside, lantadenes, verbascoside, phenolics, proanthocynidines, quercetin, safrole, β-sitosterol, campesterol, stigmasterol, β-sitosterolglucoside, α-caryophyllene, isocaryophillene, germacrene, isocaryo-phyllene, muurolene and elemene.
Uses: Leaves are cardio tonic, antiseptic, fungitoxic, antimalarial, carminative, diaphoretic, antimutagenic, antispasmodic, vermifuge and repellent. The leaves are also used to treat eczema, ulcers, fistula, bilious fever, influenza, cold, asthama, bronchitis, epilepsy, tumors, tetanus and rheumatism.
Source: Juang *et al*, 2005; Chowdhury *et al*, 2007; Qaisar *et al*, 2009; Bhakta and Ganjewala, 2009

Ocimum canum
Phyto-chemical constituents: Camphor, methyl cinnamate, citral, linalool, geraniol, limonene, 1,8-cineole, cadinene, α-pinene and α-terpineol.
Uses: The herb is antibacterial, antifungal, antiviral, analgesic, rubefacient and antidiabetic. Plant also used to cure kidney, bladder and urethra diseases, colds fevers, constipation, dysentery, tooth problems, parasitic infestations, inflammation of joints and headaches.
(Ntezurubanza *et al*, 1985; Angers *et al*, 1996; Nyarko *et al*, 2002

Petunia violacea
Phyto-chemical constituents: Solanaceous alkaloids
Uses: Leaves are used as a hallucinogen.
Source: Butler *et al*, 1981

Polyalthia longifolia
Phyto-chemical constituents: Clerodanediterpenoids, allo-aromadendrene, caryophyllene oxide, β-caryophyllene, β-selinene, α-humulene and α-curcumene.
Uses: Leaves are antibacterial, antifungal and used in the treatment of colitis, diarrhea, anorexia, fever, hypertension, helminthiasis, skin diseases, sore throat, cough and colds.
Source: Faizi *et al*, 2008; Sharker and Shahid, 2010

Pongamia pinnata
Phyto-chemical constituents: Karanjachromene, isopongachromene, pongaflavone, isopongaflavone, methylpongaglabol, pongachalcone, tetramethoxyflavone, β-sitosterol, furanoflavones, furanoflavonols, chromenoflavones, furanochalcones and pyranochalcones.
Uses: Leaves are hot, digestive, antibacterial, antifungal, acrid, analgesic, carminative, depurative, digestive, alexetetic, haematinic, laxative, styptic, anthelmintic, antiinflammatory, antiplasmodial,

antihyperglycaemics, antilipidoxidative, antidiarrhoeal, antihyperammonic and CNS depressant. Leaf infusion relieves painful rheumatic joints, tumors, decoction cures cough and expressed juice cures skin diseases, herpes, itches, piles, ulcers, abscess, dyspepsia, flatulence, gonorrhoea and leprosy.
Source: Yin *et al*, 2006; Arote and Yeole, 2010; Porwal *et al*, 2010

Salvadora persica
Phyto-chemical constituents: Dimericdihydroisocoumarin like salvadorin, benzyl nitrile, eugenol, thymol, isothymol, eucalyptol, isoterpinolene, trimetylamine and β-caryophyllene.
Uses: Leaves are bitter, corrective, deobstruent, astringent to the bowels, tonic to the liver, analgesic, anthelmintic, expectorant, carminative, diuretic, antiscorbutic, antiinflammatory and antidote. Leaves used in treatment of toothache, gum problems, skin diseases, blisters, scurvy, bronchitis, cough, asthma, rheumatism, scabies, leukoderma, teeth piles, painful tumors, haemorrhoids, gastric mucosa and kidney stones.
Source: Khalil, 2006; Sher *et al*, 2010; Khatak *et al*, 2010

MATERIALS AND METHODS

Collection and extraction of plant material: Plants used in the present study were *Ailanthus excelsa* Roxb, *Calotropis procera* Aiton, *Cassia tora* L, *Citrus limon* (L) Burm f, *Clerodendrum inerme* (L) Gaertn, *Lantana camara* L, *Ocimum canum* Sims, *Petunia violacea* Lindl, *Polyalthia longifolia* Benth & Hook F, *Pongamia pinnata* (L) Pierre, *Salvadora persica* L (Fig. 1, Table 1). The plant material for the study was collected from the campus of Gujarat University, Ahmedabad and environs. Collected plant material was washed and air dried under shade (one week). The dried plant parts were finely powdered using electric grinder, sieved (mesh size 500µ) and subjected for the extraction. All plant samples were extracted in four solvents of different polarity viz water, methanol, chloroform and petroleum ether. For aqueous extracts, powdered plant material (50 g) was extracted in 1000 ml of distilled water at 50°C temperature until the volume reduces to half. The content then filtered through whatman filter paper (no 1). The filtrate was evaporated till complete dryness in oven (40°C) (Harborne, 1984; Daniel, 1991; Patel and Jasrai, 2010). For organic solvent extraction of finely powdered plant material (100 g), in solvents like methanol, chloroform and petroleum ether, the material was soaked overnight in solvent (400ml) in air tight erlenmeyer flask. The residues were repeatedly extracted (three times) in 200 ml of solvent (Souri *et al*, 2008; Khan and Nasreen, 2010). The extracts were filtered through a whatman filter paper (no 1). The filtrate was evaporated to dryness to yield a dark-residue. Each sample was then transferred to glass vials (6 ×2 cm) and % yield of extracts were calculated (Table 2).

Table 2: Percent yield of extracts from selected plant materials extracted in different solvents

Plants	Family	Plant Part Used	% Extract Yield *			
			WT	ME	CH	PE
Ailanthus excelsa Roxb	Simaroubaceae	Twigs	28.78	11.68	12.37	1.78
Calotropis procera Aiton	Apocynaceae	Leaves	28.73	9.57	8.85	4.22
Cassia tora L	Caesalpinaceae	Aerial part	13.25	10.19	1.72	0.63
Citrus limon (L) Burm f	Rutaceae	Leaves	25.68	16.10	8.32	2.04
Clerodendrum inerme (L) Gaertn	Verbenaceae	Leaves	23.24	12.87	3.79	5.35
Lantana camara L	Verbenaceae	Twigs	45.51	20.21	5.34	2.56
Ocimum canum Sims	Labiatae	Aerial part	17.72	16.40	3.30	4.10
Petunia violacea Lindl	Solanaceae	Leaves	20.83	29.60	10.08	1.93
Polyalthia longifolia Benth & Hook F	Annonaceae	Leaves	19.93	19.74	21.64	5.12
Pongamia pinnata (L) Pierre	Fabaceae	Leaves	24.24	24.45	6.83	0.94
Salvadora persica L	Salvadoraceae	Leaves	34.22	18.38	0.99	2.23

[**Note:*** represents g extract/100g dry powder**, WT**= Water; **ME**= Methanol; **CH**= Chloroform; **PE**= Petroleum ether]

Screening antioxidant activity: The plant extracts were subjected to the antioxidant activity screening using standardized protocol (Ghasemi *et al*, 2009). The chemicals utilized were of pure and analytical grade. Readings were taken for six replicates for each sample and averages were calculated with standard errors. IC_{50} value was calculated for each standard, representing the concentration of the compounds that caused 50% inhibition/ antioxidant activity.

DPPH radical scavenging assay: 2 ml 0.5 mM methanolic solution of DPPH (1,1-Diphenyl-2-picrylhydrazyl) was mixed with the 2 ml methanolic solution containing 3 mg extract. The mixture was shaken vigorously and allowed to incubate in dark for 30 min and OD was taken at 517 nm. BHT (Butylated Hydroxy Toluene) was used as a reference compound with IC_{50} at 0.35 mg/4 ml. The calculation was performed using the formula (Ghasemi *et al*, 2009).

$$\% \text{ DPPH Radical scavenging activity (RSA)} = A\left\{\frac{\text{control -A sample}}{\text{A control}} \times 100\right\}$$

[**Note:** A control = OD of DPPH solution without extract or standard, A sample = OD of DPPH solution with extract or standard]

RESULTS AND DISCUSSION

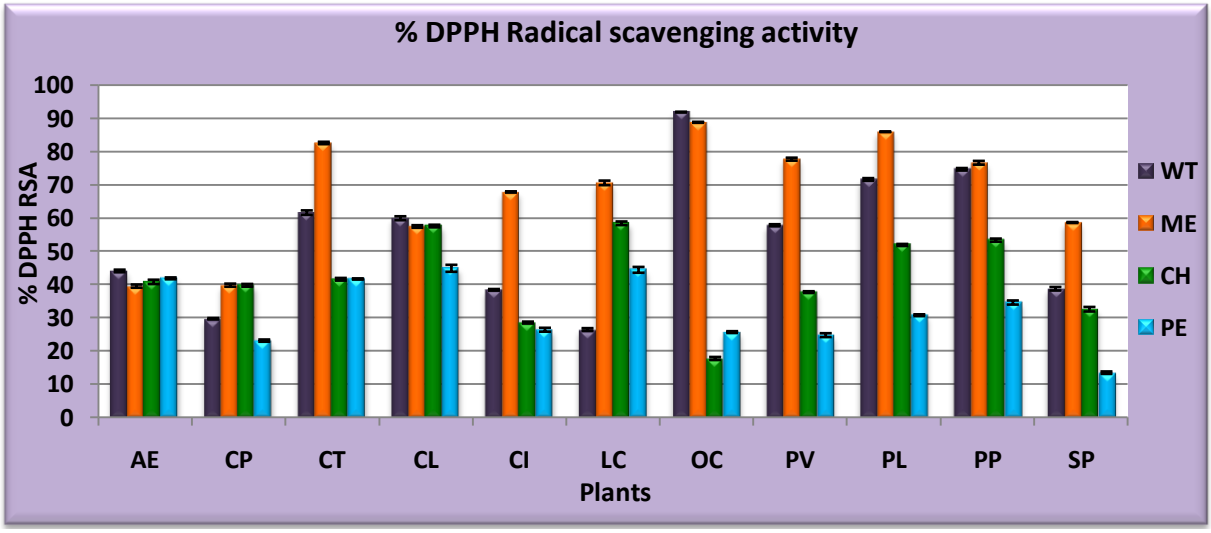

Figure 2: Antioxidant activity exhibited by plant extracts in DPPH RSA

[**Note: AE**= *Ailanthus excelsa*; **CP**= *Calotropis procera*; **CT**= *Cassia tora*; **CL**= *Citrus limon*; **CI**= *Clerodendrum inerme*; **LC**= *Lantana camara*; **OC**= *Ocimum canum*; **PV**= *Petunia violacea*; **PL**= *Polyalthia longifolia*; **PP**= *Pongamia pinnata*; **SP**= *Salvadora persica*; **WT**= Water extract; **ME**= Methanol extract; **CH**= Chloroform extract; **PE**= Petroleum ether extract]

DPPH is a nitrogen-centered free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Bhakta and Ganjewala, 2009). During this free radical scavenging assay, methanolic DPPH solution changes color from deep violet to a pale yellow due to formation of colorless α-diphenyl-β-picryl hydrazine a stable diamagnetic molecule, via either transfer of an electron or hydrogen atom to DPPH (Nahar *et al*, 2009; Sreelatha and Padma, 2009). In other words, odd electron of the radical becomes paired off with hydrogen donated by the extract and solution loses color stochiometrically depending on the number of electrons taken up, resulting in the reduction of absorption strength (Nikhat *et al*, 2009; Singh *et al*, 2009; Porwal *et al*, 2010). IC_{50} (effective concentration of extract needed for 50% free radical inhibition) is the amount of antioxidant present in the sample, necessary for 50% DPPH inhibition (Ghafar *et al*, 2010). Thus lower the IC_{50} value, indicates higher antioxidant activity.

In the present study, % DPPH RSA activity IC_{50} value for the standard BHT (was observed at 0.08 mg/ml concentration. Figure 2 illustrates a % DPPH RSA activity with reference to the scavenging ability of the extracts. Plants *Polyalthia longifolia*, *Pongamia pinnata*, *Petunia violacea*, *Lantana camara*, *Cassia tora*, *Ocimum canum*, *Citrus limon* and *Salvadora persica* extracts presented an excellent DPPH RSA activity. Marked % DPPH RSA in the range of 60 to 90% was recorded for *Cassia tora* WT and ME; *Clerodendrum inerme* ME; *Lantana camara* ME; *Ocimum canum* WT and ME; *Petunia violacea* ME; *Polyalthia longifolia* WT and ME; *Pongamia pinnata* WT and ME extracts. A significant % DPPH RSA in the range of 30 to 60% was demonstrated by *Ailanthus excelsa* WT and PE; *Cassia tora* CH and PE; *Citrus limon* WT, ME, CH and PE; *Lantana camara* CH and PE; *Petunia violacea* WT; *Polyalthia longifolia* CH and PE; *Pongamia pinnata* CH; *Salvadora persica* ME extracts (Fig. 2).

All solvent extracts demonstrated presence of an excellent antioxidant activity in the present study. Conversely comparative antioxidant activity analysis for different solvent extracts indicated best activity for the extracts prepared in solvents water and methanol compare to the chloroform and petroleum ether. This indicates the high extractive value of the polar solvents over non-polar solvents for extracting the active antioxidant phyto-chemicals from the selected plant material.

Results, clearly demonstrate that these naturally growing plants are the rich source of antioxidant compounds which proves their ability to survive in nature. Their pharmacological effects need to be explored to derive the beneficial extract fraction to be utilized for medicinal and general well-being purpose.

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