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IN-VITRO FREE RADICAL SCAVENGING POTENTIAL OF POLY HERBAL EXTRACT

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ABSTRACT

The study was designed to examine the *in-vitro* free radical scavenging potential of ethanolic polyherbal extract(EPHE).The antioxidant activity was evaluated by DPPH(1,1-diphenyl-2-picryl hydrazyl) radical scavenging activity, superoxide, hydroxyl radical, hydrogen peroxide and ferric reducing assay with reference standard Quercetin. The EPHE was found to more effective in the DPPH radical scavenging activity. The IC_{50} of EPHE and Quercetin were found to be 20.1µg/ml and 0.455µg/ml respectively. An IC_{50} value was found that EPHE of 83.61µg/ml effective in scavenging superoxide radical when compare with Quercetin (standard) 49.641µg/ml. The IC_{50} value was found to be 9.745µg/ml, 24.41µg/ml quercetin and EPHE respectively. Hence the EPHE can be considered as a good scavenger of hrdroxyl radicals. The FRAP values for the EPHE compared with Quercentin that have significant reducing power. Scavenging activity of hydrogen peroxide in 54.67µg/ml,121.8 µg/ml quercetin and EPHE respectively as reference compound was not remarkably different. It is concluded that the EPHE which exhibits high antioxidant and free radical scavenging activities.

Key words: *In-vitro*, Free radical scavenging, Poly herbal extract.

INTRODUCTION

Free radicals are undesirable products of our body's metabolic processes, caused by poor or insufficient nutrition, and from exposure to environmental pollutants. Everybody has to contend with free radicals: many are unknowingly extinguished. To control larger amounts and to limit oxidative damage, our body needs help. Nature and science have given us protection from free radicals by antioxidants. Natural antioxidant products to prevent disease and allow healing for the prevention and treatment of discomforts and disease (Anonymous 1; Anonymous 2). Free radicals and reactive oxygen species (ROS) are well known inducers of cellular and tissue pathogenesis leading to several human diseases such as cancer, inflammatory disorders, as well as in aging processes (Halliwell B, 1994; Aviram M, 2000).

Free radicals which have one or more unpaired electrons are produced during normal and pathological cell metabolites. Reactive oxygen species (ROS) react easily with free radicals to become radicals themselves. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals (O_2^-) and hydroxyl radicals (OH^-), as well as non-free radicals species (H_2O_2) and the singlet oxygen ($^1O_2$) (Adedapol AA *et al*., 2009). Antioxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and the concomitant lipid peroxidation, protein damage and DNA strand breaking (Ghosal S *et al*., 2002). Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant rich foods and the incidence of human diseases (Halliwell B, 1994). The plant *Vitex trifolia* L., (Verbenaceae) is commonly known as common chaste tree (English), *nochi* (Kannada) and *jalanirgundi* (Sanskrit). Leaves are commonly used as poultice for

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rheumatic pains, in inflammations, sprains and fever. Roots are used to treat febrifuge, painful inflammations, cough and fever. Flowers are used in treating fever and fruits in amenorrhoea. This plant is known to possess various active constituents viz., essential oil, halimane-type diterpenes, vitetrifolins and several pharmacological properties have been studied viz., antipyretic, antibacterial, against asthma and allergic diseases and the leaf extract possesses anti-cancerous, anti-oxidant activity (Orient L, 1995; Asima C; The wealth of India; Kiritikar KR and Basu BD, 1936).

Vernonia cinerea (L.) belongs to the *Asteraceae* family is an annual herb that grows in India, Bangladesh, Sri Lanka and Malay island. It is commonly known as 'little ironweed' in English, 'joanbeer', 'kukshim' in Bengali, 'puvamkurunnel' in Malayalam and 'sahadevi' in Sanskrit and Hindi. The plant is reported to be used in traditional medicine as tonic, astringent, diaphoretic, antirheumatic, anthelmintic and antidiarrheal, antimicrobial, antibacterial, antioxidant, anti-inflammatory, analgesic, antipyretic, antiflautulent,anti cancer, antispasmodic and antidiuretic properties (Arivoli S *et al*., 2011; Sreedevi A *et al*., 2011). *Ocimum basilicum* (Linn.) belongs to the *Labiatae* family. It is popularly known as "Kali Tulsi" in Hindi, is a widely grown plant of Hindus. Different parts of the plant have been claimed to be valuable in wide spectrum of diseases. It is a small perennial, tropically growing shrub of Asian origin. Medicinal plants from *Labiatae* family having anti-oxidants property. It is closely related with the prevention of degenerative illness, such as cardiovascular, neurological diseases, cancer and oxidative stress dysfunctions. A plant flavanoid has been found to be effective against ulcer in experimental animals. In our earlier studies, it has antipyretic, antiemetic, diuretic, and cardiotonic properties (Surender S, 1998; Muralidharan A, Dhanjayan R, 2004; Rekha S, Llona SV, 2002). This study reports the *in-vitro antioxidant* activity of the polyherbal extract.

MATERIALS AND METHODS

Collection and authentication of plant material

The plants *Vitex trifolia Linn.,* Leaf, *Vernonia Cinerea Linn.,* Leaf and *Ocimum basilicum Linn.,* Leaves were collected in the month of September 2010 from the surrounding area of Chennai, Tamilnadu, India. The plant material was taxonomically identified by Botanist Dr. P. Jayaraman, Director, Plant Anatomy Research Centre, Chennai, Tamil Nadu.

Preparation of polyherbal extract

The collected above plant leaves were cleaned and shade dried. The dried plant leaves were coarsely powdered and subjected to soxhlet extraction using ethanol separately. The solvent was removed by distillation under reduced pressure, which produced a greenish sticky residue (yield 10%w/w with respect to dried plant material). The equal quantity of above ethanolic leaf extracts mixed together and stored pressured air tight container, to avoid micro-organisms contamination in sterile area under controlled temperature (28±1°c).

Chemicals and reagents

DPPH(1, 1-diphenyl-2-picryl hydrazyl), DMSO, EDTA, Nitro Blue Tetrazolium (NBT), Quercentin, potassium ferricyanide, Trichloroacetic acid (TCA), Hydrogen Peroxide, Thiobarbituric Acid (TBA), Ferric Chloride, phosphate buffer, orthophosphoric acid, glacial acetic acid and all other chemicals including solvents were of AR grade and procured from Nice Chemicals Pvt. Ltd., Cochin, India.

Evaluation of free radical scavenging potential by *in-vitro* Models

DPPH radical scavenging assay

The effect of EPHE on DPPH radical was determined using the method of Liyana-Pathiranan & Shahidi. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in methanol containing 0.02–0.1 mg of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Quercentin was used as standard. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) =

$$[(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$$

Where $Abs_{control}$ is the absorbance of DPPH radical + methanol; Abs_{sample} is the absorbance of DPPH radical + sample extract/standard (Liyana-Pathiranan CM, Shahidi F, 2005).

Scavenging of Superoxide radical by Alkaline DMSO

Preparation of Test and Standard solutions

10 mg of the drug samples and the standard (Quercentin) were weighed accurately and separately dissolved in 1 mL of DMSO. These solutions were serially diluted with DMSO to obtain the lower dilutions.

Procedure

To the reaction mixture containing 1 mL of alkaline DMSO, 0.3 mL of the drug samples and standard was added in DMSO at various concentrations followed by 0.1 mL of NBT (0.1 mg) to give a final volume of 1.4 mL. The absorbance was measured at 560 nm (Elizabeth K, Rao MNA, 1990).

$$(\%) \text{ inhibition} = [(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$$

Hydroxyl Radical Scavenging Activity

The reaction mixture contained 0.1ml of deoxyribose, 0.1ml of $FeCl_3$, 0.1ml of EDTA, 0.1ml of H_2O_2, 0.1ml of ascorbate, 0.1ml of KH_2PO_4-KOH buffer and 20µl of EPHE in a final volume of 1.0ml. The mixture was incubated at 37°C for 1 hour. At the end of the incubation period, 1.0 ml of TBA was added and heated at 95°C for 20 minutes to develop the color. After cooling, the Thiobarbituric acid reactive substances (TBARS) formation was measured spectrophotometrically (Systronics, India PVT. Ltd) at 532nm against blank (water). The hydroxyl radical scavenging activity was determined by comparing the absorbance of the control with that of the samples (Halliwell B *et al*., 1987).

$$(\%)\ \text{inhibition} = [(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$$

Hydrogen peroxide scavenging activity

1.0ml of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (PBS, pH 7.4). 1.0ml of various concentrations of the EPHE or standards in methanol was added to 2ml of hydrogen peroxide solution in PBS. Then finally the absorbance was measured at 230nm after 10 minutes. The percentage inhibition was calculated using equation (Jayaprakasha GK *et al*., 2004).

$$(\%)\ \text{inhibition} = [(Abs_{control} - Abs_{sample})]/\ (Abs_{control})] \times 100$$

Ferric Reducing Antioxidant Power Assay (FRAP)

Different concentration of the EPHE was performed (20-100 µg/mL) in 0.2 M phosphate buffer pH, 6.6 containing 1% ferrocyanate. The mixture was incubated at 50 ºC for 20 minutes. 10% trichloroacetic acid (TCA, 2.5 mL) was added to a portion of this mixture (5 mL) and centrifuged at 3,000 rpm for 10 minutes. The supernatant was separated and mixed with distilled water (2.5 mL) containing 1% ferric chloride (0.5 mL). The absorbance of this mixture was measured at 700 nm. The intensity in absorbance could be the measurement of antioxidant activity of the extract (Yen GC, Duh PD, 1994).

$$(\%)\ \text{inhibition} = [(Abs_{control} - Abs_{sample})]/\ (Abs_{control})] \times 100$$

Statistical analysis

The results are expressed IC_{50} and correlation coefficient (R^2) value of FRAP. It is calculated by Using graph pad prism version 5 and correlation analysis of FRAP was carried out using the correlation and regression program.

RESULTS AND DISCUSSION

DPPH assay

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule .The reduction capability of DPPH was determined by the decrease in its absorbance at 517 nm, which is induced by anti-oxidants. On the DPPH radical (fig.1), EPH extract significant scavenging effects when compared with that Quercetin with high level of IC_{50} value of 0.4553 µg/ml, 20.1 µg/ml for quercetin and EPH extract respectively. This result is very much comparable with previous reports on DPPH assay.

Superoxide radical scavenging assay

Superoxide is a highly reactive molecule that reacts with various substances produced through metabolic processes. Superoxide dismutase enzymes present in aerobic and anaerobic organisms catalyses the breakdown of superoxide radical (Shirwaikar A, Punitha ISR, 2007). Percentage scavenging of superoxide anion examined at different concentrations of EPH extract and Quercetin (20, 40, 60, 80,100µg/ml) respectively. The percentage scavenging of superoxide radical surged with the enhanced concentration of EPH extract. The maximum scavenging activity of EPH extract and Quercetin at 100 µg/ml was found to be 57.14% and 75.39%respectively. Superoxide scavenging ability of EPH extract might primarily be due to the presence of flavanoids (Zheng W, Wang SY, 2001). The IC50 value of EPH extract and Quercetin was recorded as 83.61µg/ml and 49.64µg/ml respectively. Based on the IC50 values and percentage scavenging capacity, it was found that EPH extract is good effective in scavenging superoxide radical (fig.2).

Hydroxyl radical scavenging assay

The hydroxyl radical is the most reactive of the reactive oxygen species and it induces severe damage in adjacent biomolecules. The hydroxyl radical can cause oxidative damage to DNA, lipds and proteins. The OH scavenging activity of EPHE was assessed by its ability to compete with Quercetin for OH radicals in the OH generating/ detecting system. In the present study, the hydroxyl radical scavenging effective of the IC50 value was found to be 9.745µg/ml,24.41µg/ml quercetin and EPHE respectively (fig.3). Hence, the EPHE can be considered as a good scavenger of hydroxyl radicals.

Hydrogen peroxide radical scavenging assay

Scavenging activity of hydrogen peroxide in 121.8µg/ml of EPH extract and 54.67µg/ml of quercetin (fig.4) as reference compound was not remarkably different. The composition of hydrogen peroxide into water may occur according to the antioxidant compounds as the antioxidant component present in the EPHE was good electron donors, they may accelerate the conversion

of H_2O_2 to H_2O.

Total antioxidant power (FRAP) assay

Figure (5) shows the reducing ability of the EPHE compared with Quercentin. It was observed at 700 nm in 100 µg/ml concentration. The correlation analysis revealed that correlation exists between Quercetin and EPH extract. The correlation coefficient (R^2) for the Quercetin and EPH extract was 0.9862 and 0.9592 respectively. This result suggests that 95% of the EPH extract compared with 98% of Quercetin (STD), which have significant reducing power.

Fig 1. Effect of EPHE on 1, 1Diphenyl 2picrylhydrazyl radical scavenging activity

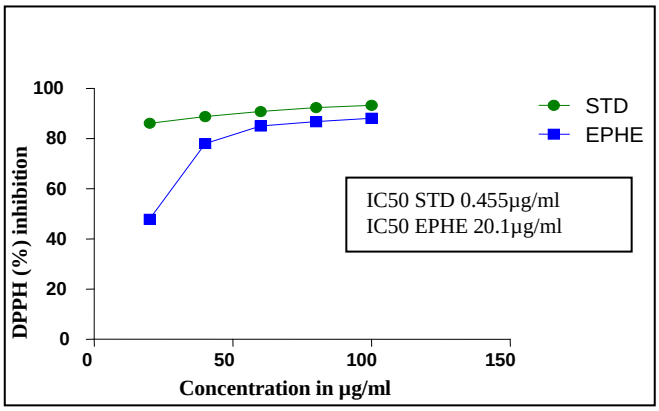


Fig 2. Effect of EPHE on Super oxide radical scavenging activity

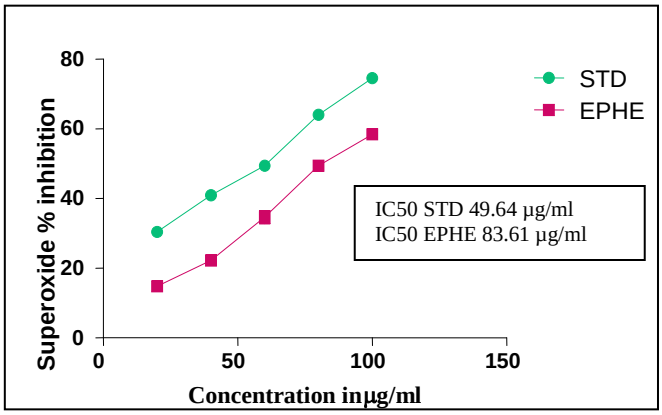


Fig 3. Effect of EPHE on Hydroxyl Scavenging activity

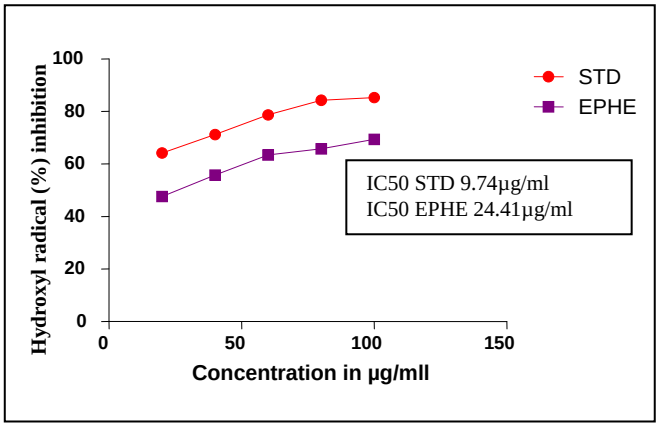


Fig 4. Effect of EPHE on Hydrogen peroxide radical scavenging activity

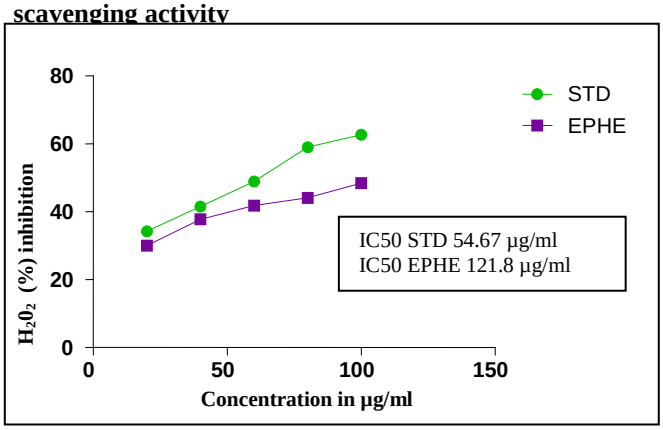
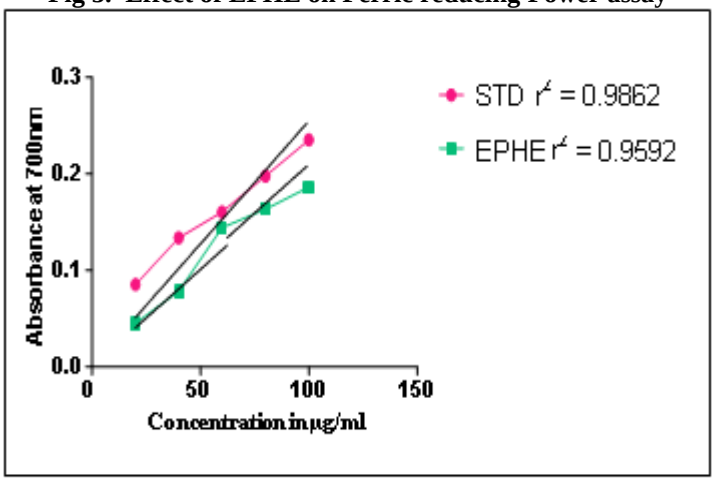


Fig 5. Effect of EPHE on Ferric reducing Power assay



CONCLUSION

From the results obtained in the present study, it is concluded that the EPHE exhibits high antioxidant and free radical scavenging activities. These *in-vitro* assays indicate that this EPHE is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Further investigations are required to find active component of these plant extracts and to confirm the mechanism of action.

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