Study on antioxidant property in selected medicinal plant extracts

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Abstract: The Clitoria ternatea, Solanum nigrum and Aloe vera leaves were extracted serially by the solvents of increasing polarity (petroleum ether, chloroform and ethanol) were tested for their free radical scavenging activity against DPPH. The petroleum ether extracts of Clitoria ternatea, Solanum nigrum and Aloe vera leaves were the most effective scavenging of DPPH. These plant extracts were also analyzed for the activities of selected enzymic antioxidants such as catalase, polyphenol oxidase and the non-enzymic antioxidants were ascorbic acid and total phenols. The results showed the leaves of Solanum nigrum possess higher levels of antioxidants.

Keywords: Petroleum ether extract; DPPH; Enzymic and Non-enzymic antioxidants; Clitoria ternatea; Solanum nigrum; Aloe vera.

Introduction

Antioxidants act as a defense mechanism that protects against oxidative damage, and include compounds to remove or repair damaged molecules. It can prevent/retard the oxidation caused by free radicals and sufficient intake of antioxidants is supposed to protect against diseases (Celiktar et al., 2007). Free radicals are not only produced naturally in the cell following a stress or respiration but also have been reported to be produced by radiation, bacterial and viral toxins, smoking, alcohol and psychological or emotional stress. The body produces many antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase, which neutralize many types of free radicals (Gamiothea-Turro et al., 2004). Oxidative stress is a factor for many human diseases, as either a cause or an effect. Plants are the source of medication for preventive, curative, protective or promotive purposes (Sidhu et al., 2007). However, the natural antioxidant compounds become important (Malpure et al., 2006). Several groups of constituents in plants have been identified as potentially health promoting in animal studies, including cholesterol lowering factors, antioxidants, enzyme inducers and others (Dragsted et al., 2006). Many of these herbal medicines are finding their way into the world market as alternatives to prescribed allopathic drugs currently available to treat various disorders and ailments (Huang et al., 2005). The rapid increase in the consumption of traditional herbal remedies worldwide has been stimulated by several observations, which have shown their use as alternative medicine. In particular cancer patients are reported to benefit from treatment with herbal medicine and survivability in many cases is significantly increased (Ho et al., 2002). These observations show herbal products to be safe, harmless, effective and free from side effects.

Methodology

Plant material

A). Clitotia ternatea (Common name- Sankupushpam) belonging to family Fabaceae is a twining shrub with alternate imparipinnate leaves. About their flowers are solitary or in pairs.

B). Solanum nigrum (Common name- night shade) belonging to family Solanaceae is a dicot weed, African paediatric plant. It is an annual branched herb of up to 90 cm high dull dark green leaves, juicy, ovate or lanceolate, and toothless to slightly toothed on the margins.
C. *Aloe vera* belonging to family Liliaceae is a succulent plant. Succulents are xerophytes which are adapted to live in the areas of low water availability and are characterized by possessing a large water storage tissue. Although pulp is likely water storage tissue.

**Rapid screening of antioxidant activity by Dot plot assay (Mensor et al. 2001)**

Antioxidants react with diphenyl-1-picryl hydrazyl (DPPH) and convert it to diphenyl-picryl hydrazine. The degree of discolouration from purple to yellow colour can be used as a measure of the scavenging potential of antioxidant extract. Aliquots (3µl) of *Clitoria ternatea* extracts were spotted on a TLC plate and allowed to dry. The TLC plate bearing the dry spots was placed upside down for 10 seconds in the solution of DPPH. The spots exhibiting radical scavenging, antioxidant activity showed up as yellow spots in a violet background. The intensity of the yellow colour depends on the amount and nature of the radical scavenger present in the spot.

**DPPH photometric assay by Soler-Rivas et al. (2000)**

The leaf extracts were tested for their scavenging activity against the stable free radical DPPH (2, 2'-diphenyl-1-picryl hydrazyl). The ability of the leaf extracts to bleach DPPH can be quantified using a spectrophotometric assay, the extent of scavenging causing a proportionate change in the absorption at 518nm. An exact amount (0.5ml) of the methanolic solution of DPPH was added with 20 µl of the leaf extracts in the different solvents and the crude aqueous extract (corresponding to 4mg) and 0.48ml of methanol, and allowed to stand at room temperature for 30 minutes. Methanol served as the blank. After 30 minutes, the absorbance was measured at 518nm and converted into percentage radical scavenging activity as follows

\[
\text{Scavenging activity (\%)} = \frac{A_{518} \text{ [sample]} - A_{518} \text{ [blank]}}{A_{518} \text{ [blank]}} \times 100
\]

**Enzymic antioxidants**

The enzymic antioxidants analyzed in the leaves were Catalase and Polyphenol oxidase.

**Assay of catalase**

Catalase activity in the leaves of white and blue variety of *Clitoria ternatea*, *Solanum nigrum* and *Aloe vera* were assayed by the method of Luck (1974). The UV light absorption of hydrogen peroxide can be easily measured at 240nm. On decomposition of hydrogen peroxide by catalase, the absorption decreases with time. The enzyme activity can be arrived at from this decrease. An exact amount (0.5g) of *Clitoria ternatea*, *Solanum nigrum* and *Aloe vera* leaves were homogenized in 2.5ml of phosphate buffer. An aliquot (3.0ml) of hydrogen peroxide in phosphate buffer was taken and the baseline was adjusted at 240nm in a spectrophotometer. The enzyme extract (40 µl) was rapidly added and mixed thoroughly. The time interval required for a decrease in absorbance by 0.05 units were recorded at 240nm. The concentration of H₂O₂ was calculated using the extinction coefficient 0.036 per µM cm⁻¹. One unit is the amount of enzyme activity required to decrease the absorbance at 240nm by 0.05 units.

**Assay of polyphenol oxidases (ppo)**

Polyphenol oxidase activity was determined by the method of Esterbauer et al. (1977). In this method, catechol oxidase and laccase activities were simultaneously estimated spectrophotometrically. Phenol oxidases are copper proteins, which catalyse the aerobic oxidation of certain phenolic substances to quinones, which are auto oxidized to dark brown pigments generally known as melamins, which can be assayed spectrophotometrically at 495nm. *Clitoria ternatea*, *Solanum nigrum* and *Aloe vera* leaves (0.5g) were homogenized in the medium, containing Tris-HCl, sorbitol and NaCl, and was made up to 2.0ml. The homogenate was centrifuged and the supernatant was used for the assay. An aliquot of 2.5ml of (0.1M) phosphate buffer and 0.3ml of catechol solution were added into a cuvette and the spectrophotometer was set at 495nm. The enzyme
extract (0.2ml) was added and the change in absorbance was recorded for every 30 seconds up to 5 minutes. One unit of either catechol oxidase or laccase is defined as the amount of enzyme that transforms one µmole of dihydrophenol to one µmole of quinone/minute.

The activity of PPO can be calculated using the formula

\[ \text{Enzyme unit} = K \times (\frac{\Delta A}{\text{min}}) \]

- K for catechol oxidase = 0.272
- K for laccase = 0.242

**Non-enzymic antioxidants**

The non-enzymic antioxidants analyzed in the *Clitoria ternatea*, *Solanum nigrum* and *Aloe vera* leaves were ascorbic acid and total phenols.

**Estimation of ascorbic acid**

Ascorbic acid, a scavenger of oxyradicals was estimated by the method of Roe and Keuther (1943). Ascorbate is converted to dehydroascorbate by the treatment with activated charcoal. Dehydroascorbic acid then reacts with 2,4-dinitrophenyl hydrazine to form osazones, which dissolve in sulphuric acid to give an orange coloured solution, whose absorbance can be measured spectrophotometrically at 540nm. An accurate amount (1g) of leaves were homogenized in 4% TCA and made upto 10ml. Centrifuged and the supernatant obtained was treated with a pinch of activated charcoal, mixed vigorously and kept for 10 minutes. Centrifuged again to remove the charcoal residue and the supernatant obtained was used for the estimation. Aliquots of 0.5 - 1.0ml of this supernatant were taken and 0.2 to 1.0ml of standard ascorbate were made up to 2.0ml with 4% TCA. 0.5ml of DNPH reagent was added to all the tubes, followed by 2 drops of 10% thiourea solution. The contents were mixed and incubated at 37°C for 3 hours. The osazones formed were dissolved in 2.5ml of 85% sulphuric acid, in cold. To the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid. After incubation for 30 minutes at room temperature, the absorbance was read spectrophotometrically at 540nm. From the standard curve constructed on an electronic calculator set to the linear regression mode, the concentration of ascorbate in the samples were calculated and expressed as mg ascorbate/g leaf.

**Estimation of total phenols**

Total phenols were assayed by the method proposed by Mallick and Singh (1980) in plant tissue. Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium to produce a blue-coloured complex which can be quantified spectrophotometrically at 650nm. An accurate amount (0.5g) of the leaves was homogenized in 10X volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The residue was reextracted with 80% ethanol. The supernatants were centrifuged, pooled and evaporated to dryness. The residue was dissolved in a known volume of distilled water and 0.5ml of Folin-Ciocalteau reagent was added to it. After 3 minutes, 2.0ml of 20% sodium carbonate solution was added, mixed thoroughly and placed in a boiling water bath for exactly 1 minute, cooled and measured the absorbance at 650 nm in a spectrophotometer. Standard catechol solution (0.2-1ml) corresponding to 2.0-10 µg concentrations were added with Folin-Ciocalteau reagent and sodium carbonate. A standard curve was constructed using an electronic calculator on the linear regression mode, using which the concentrations of phenols in the samples were read. The values are expressed as mg phenols/g leaf.

**Statistical analysis of the data obtained**

All the parameters studied were subjected to statistical treatment using online statistical package was adapted to all the parameters.

**Results**

**Dot plot assay**

The dot plot assay as shown in Figure 1 has the maximum DPPH scavenging activity was expressed by the petroleum ether extract, followed by ethanolic and chloroform extracts as shown in Figure-1.
Clitoria ternatea leaves - Dot plot assay

Solanum nigrum leaves - Dot plot assay

Aloe vera leaves - Dot plot assay

Figure 1: Total antioxidant activity of Clitoria ternatea, Solanum nigrum and Aloe vera leaves by Dot plot assay.

Photometric assay

To confirm the observations in dot plot, radical scavenging effects of Clitoria ternatea, Solanum nigrum and Aloe vera leaves were also quantified using DPPH photometric assay are represented in Figure 2.

Figure 2: Total antioxidant activity of Clitoria ternatea, Solanum nigrum and Aloe vera leaves by DPPH method.

Levels of enzymic and non-enzymic antioxidants

The enzymic antioxidants such as catalase and polyphenol oxidase (Figure 3) and non-enzymic antioxidants such as ascorbic acid and total phenols (Figure 4) were analyzed in the leaves of Clitoria ternatea, Solanum nigrum and Aloe vera. The results were found to be good sources of enzymic and non-enzymic antioxidants in all the above selected plants but in Solanum nigrum leaves had higher level of activities when compared to others.

Figure 3: Enzymic antioxidant activity of Clitoria ternatea, Solanum nigrum and Aloe vera leaves.

Figure 4: Non-enzymic antioxidant activity of C. ternatea, S. nigrum and A. vera leaves.
Discussion

Plant extracts and their isolated constituents have always been an important part of various therapeutic systems (Vanitha and Kathiravan, 2006). Many medicinal plants have been analyzed and reported for their DPPH scavenging activity. The DPPH scavenging ability of medicinal plants has been attributed to several components, including phenolics (Bartolome et al., 2003), flavonoids (Shetgiri and D'Mello, 2003). Rosemarinic acid and luteolin isolated from Perilla frutescens var acuta showed significant DPPH scavenging ability (Gu et al., 2009). Acokanthera oppositifolia Lam and Adenia gumnifera Harv effectively scavenged DPPH (Adedapo et al., 2008). A comparative study of antioxidant levels in different Piper species showed a differential antioxidant status with reference to both enzymic and non-enzymic antioxidants (Karthikeyen and Rani, 2003). The composition of antioxidants varies widely with several factors like the variety, climatic conditions, part of the plant analyzed, post harvest handling, processing and storage (Rodriguez-Amaya, 2003). Coffea arabica and Coffea canephora of green coffee samples has been reported to have high catalase activity (Montavon et al., 2007). In recent studies, Teucrium polium. L (Sharfifar et al., 2009), whilst rush crumps (Conforti et al., 2009) and raspberries (Wange et al., 2009) have been shown to possess higher level of phenols and flavonoid content. In our study, Solanum nigrum leaves showed the maximum antioxidant content when compared to others.

Conclusion

The Clitoria ternatea, Solanum nigrum and Aloe vera leaves, extracted serially into solvents of increasing polarity (petroleum ether, chloroform, ethanol) were tested for their free radical scavenging activity against DPPH. The petroleum ether extracts of Solanum nigrum leaves were the most effective scavengers of DPPH radicals than Clitoria ternatea and Aloe vera leaves. Based on the above results, in order to reap the maximum benefits petroleum ether extract of the leaves was prepared and used in the subsequent studies. The effects of enzymic and non-enzymic antioxidants was analysed in the leaves of Clitoria ternatea, Solanum nigrum and Aloe vera. The results showed that the Solanum nigrum possess higher levels of enzymic and non-enzymic antioxidants. The present study, thus, scientifically validates and strengthens the candidature of Clitoria ternatea, Solanum nigrum and Aloe vera leaves in the preparation of medicinal aids to combat the wide spectrum of myriad diseases arising due to oxidative stress.

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References


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