Study on Antioxidant and Antimicrobial Activities of the Selected Medicinal Plants

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Abstract

The selected medicinal plants were investigated for the pharmaceutical uses such as to act as antioxidant agents. The Clitoria ternatea, Solanum nigrum and Aloe vera leaves were extracted serially by the solvents of increasing polarity (petroleum ether, chloroform, butanol, ethanol and acetone) were tested for their free radical scavenging activity against DPPH by using 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 peroxidase and reduced glutathione and the non-enzymic antioxidants were total carotenoids, flavanoids ant total chlorophyll. The results showed the leaves of Solanum nigrum possess higher levels of antioxidants. The petroleum ether, chloroform, ethanol, and acetone extracts of Clitoria ternatea, Solanum nigrum and Aloe vera leave were studied for their antimicrobial activity against selected Gram-positive and Gram-negative bacteria using agar well diffusion method. The extracts showed varied levels of antimicrobial activity against the tested pathogens.

Key words: 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 used to protect against diseases. 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, that neutralize many types of free radicals. 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. However, the natural antioxidant compounds become important. 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. 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. 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. These observations show herbal products to be safe, harmless, effective and free from side effects. Many of these classes of phytochemicals in herbal medicine are finding therapeutic use. In particular cancer patients are reported to benefit from treatment with herbal medicine and survivability in many cases is significantly increased.

Materials & Methods

Plant material

A) *Clitoria ternatea* habit is a twining shrub with alternate imparipinnate leaves. About their flowers are solitary or in pairs. The classification of *Clitoria ternatea* is given below.

Family: Fabaceae
Subfamily: Faboideae
Genus: *Clitoria*
Species: *ternatea*
Botanical name: *Clitoria ternatea.*
Common name: Sankupushpam this is plant description.

B) *Solanum nigrum* habit dicot weed in solanaceae family it is a African pediatric plant used for several ailments that are responsible for to infant mortality especially to convulsions. 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. The classification of *Solanum nigrum* is given below.

Family: Solanaceae
Genus: *Solanum*
Species: *nigrum*
Botanical name: *Solanum nigrum*
Common name: night shade this is plant description.

*Aloe vera* habit it 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. The classification of *Aloe vera* is given below.

Family: Liliaceae
Genus: *Aloe*
Species: *vera*
Botanical name: *Aloe vera* this is plant description.

Sample collection:

The leaves of the three medicinal plants were obtained from the local market of Madurai. The obtained specimens were washed twice with tap water followed by rinsing in distilled water twice, then dried under shade for 5-7 days. The dried specimen was powdered and used for the further studies.

The same plant specimens were subjected to 1% SDS treatment followed by 0.01% mercuric chloride treatment for the preparation of plant specimen in m Herbarium sheets to get proper autheciation.
Rapid screening of antioxidant activity by Dot plot assay

Principle
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 extracts. It should not be In procedure.

Reagents
TLC plates (Silica gel 60 F254, Merck), DPPH (0.4mM in methanol)

Procedure
Aliquots (3µl) of Clitoria ternatea, Solanum nigrum and Aloe vera leaves 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

Principle
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. It should not be In procedure

Reagents
DPPH (0.3 mM in methanol), Methanol write all reagents under one heading

Procedure
An exact amount (0.5ml) of the methanolic solution of DPPH was added with 20 µl of the Clitoria ternatea, Solanum nigrum and Aloe vera leaves (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:

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

Enzymic Antioxidants

Assay of superoxide dismutase (SOD)

Superoxide dismutase activity was assayed by the method of Kakkar et al. (1984).

Reagents
Sodium pyrophosphate buffer (0.025M, pH 8.3), Phenazine methosulphate (PMS) (186 µM), Nitroblue tetrazolium (NBT) (300 µM), NADH (780 µM), Glacial acetic acid, n-butanol Potassium phosphate buffer (50mM, pH 6.4)

Procedure
Preparation of enzyme extract
An accurate amount (0.5g) of leaves of Clitoria ternatea, Solanum nigrum and Aloe vera leaves was homogenized with 3.0ml of potassium phosphate buffer, centrifuged at 5000 rpm for 10 minutes and the supernatant was used for the assay.

Assay
The assay mixture contained 1.2ml of sodium pyrophosphate buffer, 0.1ml of PMS, 0.3ml of NBT, 0.2ml of enzyme preparation and water in a total volume of 2.8ml. The reaction was started by the addition of 0.2ml of NADH. After incubation at 30˚C for 90 seconds, the reaction was stopped by the addition of 1.0ml of glacial acetic acid. The reaction mixture was stirred vigorously with 4.0ml of n-butanol. The mixture was allowed to stand for 10 minutes and then centrifuged. The intensity of the chromogen in the butanol layer was measured at 560 nm against butanol as blank. The reaction mixture devoid of enzyme served as control.

One unit of enzyme activity is defined as the enzyme reaction that gave 50% inhibition of NBT reduction in one minute.
**Assay of peroxidase**

Peroxidase activity was assayed spectrophotometrically by the method of Reddy *et al.* (1995).

**Reagents**

Pyrogallol (0.05M in 0.1M phosphate buffer, pH 6.5), H$_2$O$_2$ (1% in phosphate buffer, pH 6.5)

**Procedure**

**Preparation of enzyme extract**

An accurate amount (0.5g) of *Clitoria ternatea, Solanum nigrum* and *Aloe vera* leaves was homogenized in 2.5ml of 0.1M phosphate buffer, centrifuged and the supernatant was used as the enzyme source.

**Assay**

Pyrogallol solution (3.0ml) and enzyme extract (0.2ml) were pipetted out into a cuvette. The spectrophotometer was adjusted to read ‘zero’ at 430nm. H$_2$O$_2$ (0.5ml) was added to the test cuvette. The change in absorbance was recorded for every 30 seconds upto 3 minutes. One unit of peroxidase activity is defined as the change in absorbance per minute at 430 nm.

**Non Enzymic Antioxidants**

**Estimation of total carotenoids**

The method described by Zakaria *et al.* (1979) was followed for the estimation of total carotenoids.

**Reagents**

Petroleum ether (60-80°C), Anhydrous sodium sulphate, Alcoholic potassium hydroxide (12%)

**Procedure**

An exact amount (0.5g) *Clitoria ternatea, Solanum nigrum* and *Aloe vera* leaves *Clitoria ternatea, Solanum nigrum* and *Aloe vera* leaves was homogenized and saponified for about 30 minutes in a shaking water bath at 37°C with a specific volume of 12% alcoholic KOH. The saponified extract was transferred into a separating funnel containing 10 to 15ml of petroleum ether (40-60°C) and mixed well. The lower aqueous phase was transferred to another separating funnel and collected the upper petroleum ether containing the carotenoid pigment. The extraction was repeated until the aqueous phase was colourless. To the petroleum ether extract, a small quantity of anhydrous sodium sulphate was added to remove turbidity. The absorbance of the extract at 450nm was noted in a spectrophotometer using petroleum ether as blank.

The amount of total carotenoids was calculated using the formula,

$$A_{450} \times \text{Volume of the sample} \times 100 \times 4$$

Amount of total carotenoids

Weight of the sample

The total carotenoids were expressed as mg/g leaf.

**Estimation of flavonoids**

Flavonoids were extracted and estimated by the method of Cameron *et al.* (1943).

**Reagents**

Vanillin reagent (1% in 70% H$_2$SO$_4$), Catechin standard (110 µg/ml).

**Procedure**

**Extraction**

Take (0.5g) *Clitoria ternatea, Solanum nigrum* and *Aloe vera* leaves were extracted first with MeOH:H$_2$O (2:1) and secondly with MeOH:H$_2$O (1:1). The two extracts were then combined and evaporated to about 1/3 of the original volume or until most of the MeOH had been removed. The resultant aqueous extract was cleared of low polarity contaminants such as fats, terpenes, chlorophylls and xanthophylls by extraction with hexane or chloroform. This was repeated several times and the extracts combined. The solvent extracted aqueous layer containing the bulk of the flavonoids was then concentrated and used for the assay. Write all extraction methods under one heading.
Estimation:
An aliquot of the extract was pipetted out and evaporated to dryness. 4.0ml of vanillin reagent was added and heated for 15 minutes in a boiling water bath. The standard was also treated in the same manner. The optical density was read at 340nm. The values are expressed as mg flavonoids/g leaf.

Estimation of reduced glutathione

Estimation of reduced glutathione was done according to the procedure described by Moron et al. (1979).

Principle
Reduced glutathione (GSH) is measured by its reaction with DTNB (5, 5'-dithiobis nitro benzoic acid) [Ellman’s reaction], to give a yellow coloured compound that absorbs at 412 nm.

Reagents
TCA (5%), Sodium phosphate buffer (0.2M, pH 8.0), DTNB solution (0.6 mM in 0.2M phosphate buffer), Standard glutathione (10 nmoles/ml of 5% TCA)

Procedure
An accurate aliquot (0.1 ml) of the supernatant was made up to 1.0 ml with 0.2M sodium phosphate buffer. Freshly prepared DTNB solution (2.0 ml) was added and the intensity of the yellow colour formed was read at 412nm in a spectrophotometer after 10 minutes. A standard curve of GSH was prepared between the concentration ranges of 2 to 10 nmoles. The values are expressed as nmoles GSH/g leaf.

Estimation of chlorophyll

The estimation of chlorophyll was done according to the procedure described by Witham et al. (1971).

Principle
Chlorophyll is extracted in 80% acetone and the absorption at 663nm and 645nm are read in a spectrophotometer. Using the absorption coefficients, the amount of chlorophyll is calculated.

Reagent
80% acetone.

Procedure
An accurate amount (1g) of Clitoria ternatea, Solanum nigrum and Aloe vera leaves were extracted with 20ml of 80% acetone, centrifuged (5000rpm for 5 minutes) and transferred the supernatant to a 100ml volumetric flask. This procedure was repeated until the residue was colourless. The supernatant was made upto 100 ml with 80% acetone. The absorbance of the solution was read at 645nm and 663nm against 80% acetone blank. The amount of chlorophyll present in the extract was calculated using the following formula:

\[
\text{Total chlorophyll} = 20.2(A_{645}) + 8.02 (A_{663}) \times \frac{V}{1000 \times W}
\]

where V was the final volume of the extract and W was the fresh weight of the leaves taken for extraction. The results are expressed as mg chlorophyll/g leaf.

Antibacterial activity

The antimicrobial test was performed by following agar disc diffusion method and well diffusion method using Mueller Hinton Agar No. 2 medium. Microbial growth was determined by measuring the diameter of the inhibition zone.

Results

Dot plot assay
The dot plot assay has the maximum DPPH scavenging activity was expressed by the petroleum ether extract followed by ethanol, chloroform, butanol extracts as shown in Plate 1a,b and c.
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 1.

Levels of enzymic and non-enzymic antioxidants
The enzymic antioxidants such as superoxide dismutase, Peroxidase (Figure 2 a and b) and Non-enzymic antioxidants such as carotenoids, flavanoids, reduced glutathione and chlorophyll (Figure 3 a, b, c and d) 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 compare to others.

Antibacterial activity
The results for antibacterial activity revealed that the three medicinal plant extracts posses good activity and the zone of inhibition was observed in the range of 11 -19 mm.

Plate 1a
Dot plot assay of Clitoria ternatea leaf extracts

Plate 1b
Dot plot assay of Solanum nigrum leaf extracts

Plate 1c
Dot plot assay of Aloe vera leaf extracts
Figure 1: Estimation of total antioxidant activity by DPPH photometric assay

Figure 2a: Estimation of Superoxide dismutase

Figure 2b: Estimation of peroxidase
Figure 3a: Estimation of Carotenoids

![Estimation of carotenoids](chart)

Figure 3b: Estimation of Flavanoids

![Estimation of flavanoids](chart)

Figure 3c: Estimation of Reduced glutathione

![Estimation of Reduced glutathione](chart)
Figure 3 d: Estimation of Chlorophyll

Figure 4 a: Antibacterial activity of *Clitoria ternatea* leaf extracts

Figure 4 b: Antibacterial activity of *Solanum nigrum* leaf extracts
**Discussion**

The importance of free radicals and ROS has attracted increasing attention over the past decade\(^{16}\). The reactivity of free radicals varies with many changes, causing inflammation or damage, especially to DNA, lipids and proteins. They have been implicated in human diseases such as lung diseases, heart failure, nephrotoxicity, inflammation, and diabetes \(^{17}\). Plant extracts and their isolated constituents have always been an important part of various therapeutic systems \(^{18}\). The use of different parts of the medicinal plants not only helps to decrease the cost of medication but is also locally available with lesser side effects as compared to chemical-based formulations \(^{19}\). The use of plant extracts and phytochemicals can be of great significance in therapeutic treatments \(^{20}\). A comparative study of antioxidant levels in different Piper species showed a differential antioxidant status with reference to both enzymic and non-enzymic antioxidants \(^{21}\). Our results also showed that variations exist in the levels of antioxidants among the two different varieties. 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 \(^{22}\). It has also been reported that the antioxidant capacity confers high tolerance to a range of environmental stresses and is important in breeding programs, aimed to improve plant protection \(^{23}\). Thus, the antioxidant systems in plants have the potential to increase agricultural production and quality with implications for the regulation of photosynthesis \(^{24}\). It has been reported that the components of both the enzymic and the non-enzymic antioxidants system correlate well with oxidative stress during senescence and during plant development. SOD and CAT activities declined in *Triticum aestivium* leaves upon senescence \(^{25}\). Coffea arabica and Coffea canephora of green coffee samples has been reported to have high catalase activity \(^{26}\). *Rhinacanthus nasutus* leaves have been reported to be the richest source of both enzymic and non-enzymic antioxidants among three underexploited medicinal plants \(^{27}\).

**References**