

Identification of Chemical Constituent of Combine Sample of Some Medicinal Plants for Antioxidant Activity

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ABSTRACT

The use of Natural products as medicine has been practiced from ancient time in World. With an estimation of WHO that as many as 80% of world's population rely on herbal traditional medicines as their primary health care. In recent years the interest to evaluate plants possessing antioxidant activity for various diseases is growing. Based on local use of common diseases and ethno-botanical knowledge, an attempt has been made to investigate the antioxidant activity of combine extract of Aloe barbandesis (leaves), Tinospora cordifolia (stem), Triticum aestivum (straw) Azadirachta indica (leaves) and Ocimum sactum (leaves). In this study the combine extract of selected plant materials, were analysed for their antioxidant activity by peroxide value method and diphenylpicrylhydrazyl radical scavenging method along with the reference sample ascorbic acid. The extract were further analysed to determine their total phenolic content by Folin-Ciocalteau method and total flavonoid content by Dowd method along with the reference sample rutin and gallic acid respectively. The extract exhibited significant antioxidant activity, total phenolic and flavonoid content. The extract can be used effectively for medication purposes.

Keywords: Medicinal plants, phytochemical constituent, Antioxidant activity.

INTRODUCTION

Medicinal plants are important sources for biologically active drugs [1]. About 1500 medicinal plants have been identified in Indian system, out of which 500 species are mostly used in the preparation of drugs. Medicinal plants containing active chemical constituents with high antioxidant property play an important role in the prevention of various degenerative diseases [2] and have potential benefits to the society. The herbs are natural, therefore they must be harmless. Due to this harmless nature, natural antioxidants from plant sources are safe [3].

Tinospora cordifolia (part used stem) belongs to the family *Menispermaceae* is used as an ingredient for Ayurvedic preparation in general debility, dyspepsia, fevers and urinary diseases [4]. *Aloe barbadensis* belongs to the *Liliaceal* family. Aloe vera contains over 75 nutrients and 200 active compounds, including vitamins, enzymes, minerals, sugars, lignin, anthraquinones, saponins, salicylic acid and amino acids [5]. Numerous scientific studies on Aloe vera are demonstrating its analgesic, anti-inflammatory, wound healing, immune modulating and anti-tumor activities as well as antiviral [6]. *Triticum aestivum* belongs to family *Gramineae* is an easily grown plant, the plant has anticancer and other pharmacological properties. They are used in the treatment of malaise, sore throat, abdominal coldness, constipation and cough [7]. *Azadirachta indica* belongs to family *Meliaceae* distributed widespread in the world. The chemical constituents contain many biologically active compounds that can be extracted from *azadirachta indica*, including alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones. Neem leaf is effective in treating eczema, ringworm etc. *Ocimum sactum* belongs to the family

Labiatae. The leaves yield an essential oil containing eugenol, carvacol, methyl eugenol and possess various medicinal properties [8].

Although a lot of works have been carried out on the above consider plants, but there is still important information on the combine effect of different parts of these useful medicinal plants. This study was designated to screen the different medicinal plants for antioxidant activity.

MATERIALS AND METHODS

Collection of Plant Material and Preparation of Extracts

Fresh leaves of *Azadirachta indica*, *Ocimum sactum*, *Aloe barbandesis*, Stem of *Tinospora cordifolia* were collected from their proper origin. The separate plant materials were air-dried at room temperature (26°C) for 2 weeks, after which it was grinded to a uniform powder. The mixture of acetone and methanol (30:70) extract were prepared by using 75 g each of the dry powdered plant materials in soxhlet apparatus at 40°C for 48 h. The extract were filtered after 48 h, the extracts were concentrated using a rotary evaporator with the water bath set at 40°C. The percentage yield of extracts ranged from 7-17% w/w. The extract of each plant material were mixed in equal proportion and also make a combine sample of different extract.

Phytochemical screening

The combine extract was subjected to qualitative phytochemical screening for reducing sugar, terpenoids, cardiac glycoside saponin, alkaloids, tannin and flavanoids according to the method of Harbone [9].

Table I: Phytochemical constituents of combine extract of *Azadirachta indica*, *Ocimum sactum*, *Aloe barbandesis*, *Tinospora cordifolia* and *Triticum aestivum*

| S.No. | Phytochemical components of qualitative analysis | Combine extract |
|-------|--|-----------------|
| 1. | Reducing sugar | + |
| 2. | Terpenoids | + |
| 3. | Cardiac glycoside | + |
| 4. | Saponin | + |
| 5. | Alkaloids | + |
| 6. | Tannin | + |
| 7. | Flavanoids | + |

+ = presence

Determination Antioxidant activity

Antioxidant activity of combine extract of above mentioned plants was determined against hydrogen peroxide and 2, 2-diphenyl-1-picrylhydrazyl (DPHH) radical scavenging along with the reference sample Ascorbic acid. The extract were further analysed to determine their total phenolic content by Folin-Ciocalteu method and total flavonoid content by Dowd method along with the reference sample gallic acid and rutin respectively.

Determination of the total flavonoid content (TFC)

For TFC different concentration of plant extract (1mg/ml) and different concentration of rutin as a standard were taken. 0.5ml of sample solution was taken and 2.5ml of distilled water and 0.150ml of 5% NaNO₂ was added. After 6 min, 75 µl of 10% AlCl₃ was added. After another 5 min, 0.5 ml of 1M NaOH was added to the mixture. Immediately, the absorbance of the mixture was determined at 510 nm *versus* prepared water blank [10].

Determination of the total phenolic content (TPC)

The amount of total phenolic in extracts was determined with the folin ciocalteu reagent. Galic acid was used as a standard and the total phenolic were expressed as mg/g gallic acid equivalent (GAE). Concentration of 0.01 to 0.1, mg/ml of gallic acid were prepared in methanol. Concentration 1mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2.5ml of a 10 fold dilute folin-Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was at read at 760 nm spectrometrically. The folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, there by producing a blue colour upon reaction. This blue colour is measured spectrophotometrically. Thus total phenolic content can be determined [11].

DPPH scavenging activity

1mg of extract powder were dissolved in 1 ml of 90% methanol solution to obtain 1000 µg/ml sample solutions were series diluted in to concentration ranging from 400-1000 µg/ml (i.e. 400, 500, 600, 700, 800, 900 and 1000 µg/ml). 200 µM solution of DPPH in methanol was prepared and 1.5 ml of this solution was added to 1.5 ml of methanol extract solution at different concentrations (400-1000 µg/ml). Mixture of methanol and DPPH were used as the standard control. Thirty minutes later, the absorbance was measured at 517 nm. The absorbance of DPPH solution decreases when kept in contact with antioxidant test sample and free radical scavenging activity is inversely proportional to the absorbance of DPPH solution [12, 13]. Percent inhibition of DPPH free radical scavenging activity was calculated using the following formula. Ascorbic acid was used as a standard control. IC₅₀ values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

$$\text{DPPH Scavenged (\%)} = (A_{\text{cont}} - A_{\text{test}}) / A_{\text{cont}} \times 100$$

Where A_{cont} is the absorbance of the control reaction.

A_{test} is the absorbance in the presence of the sample of the extract.

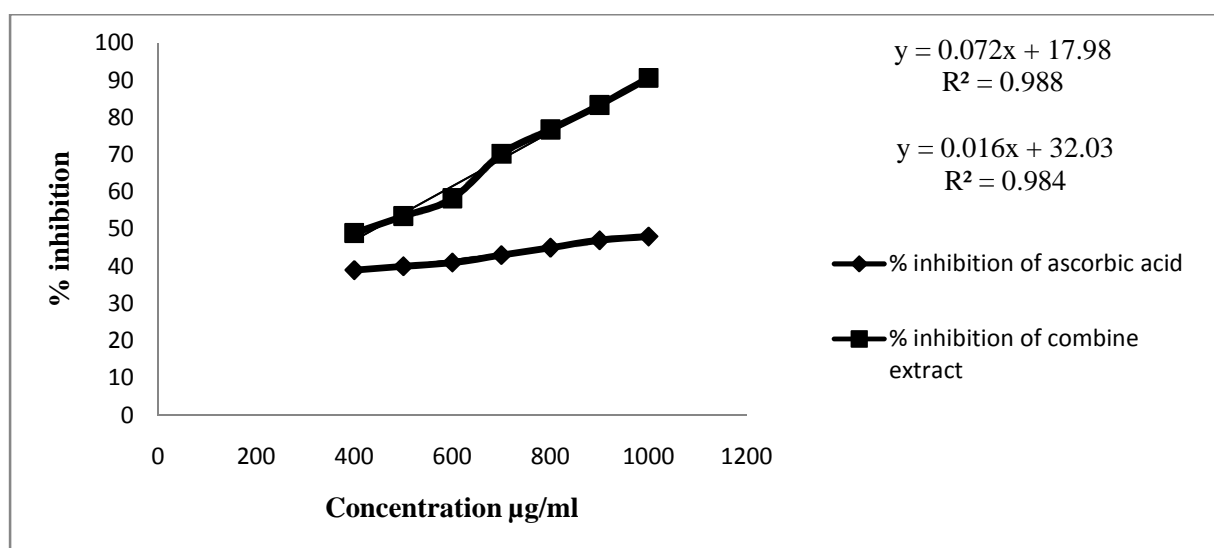


Figure I: Determination of IC₅₀ values for standard and combine extract from linear correlation between concentrations (µg/ml) verses percentage of scavenging of DPPH.

Scavenging of hydrogen peroxide

Sample with different concentration (i.e. 400, 500, 600, 700, 800, 900 and 1000 µg/ml) were added to 0.1 M phosphate buffer solution (pH 7.4, 3.4 ml) respectively and mixed with 43mM H₂O₂ solution (0.6 ml). After 10 minutes, the reaction mixture absorbance was determined at 230 nm. The reaction mixture without sample was used as the blank [11]. Ascorbic acid was used as a standard control.

$$\text{The \% inhibition activity} = (A_{\text{cont}} - A_{\text{test}}) / A_{\text{cont}} \times 100$$

Where A_{cont} is the absorbance of the control reaction.

A_{test} is the absorbance in the presence of the sample of the extract.

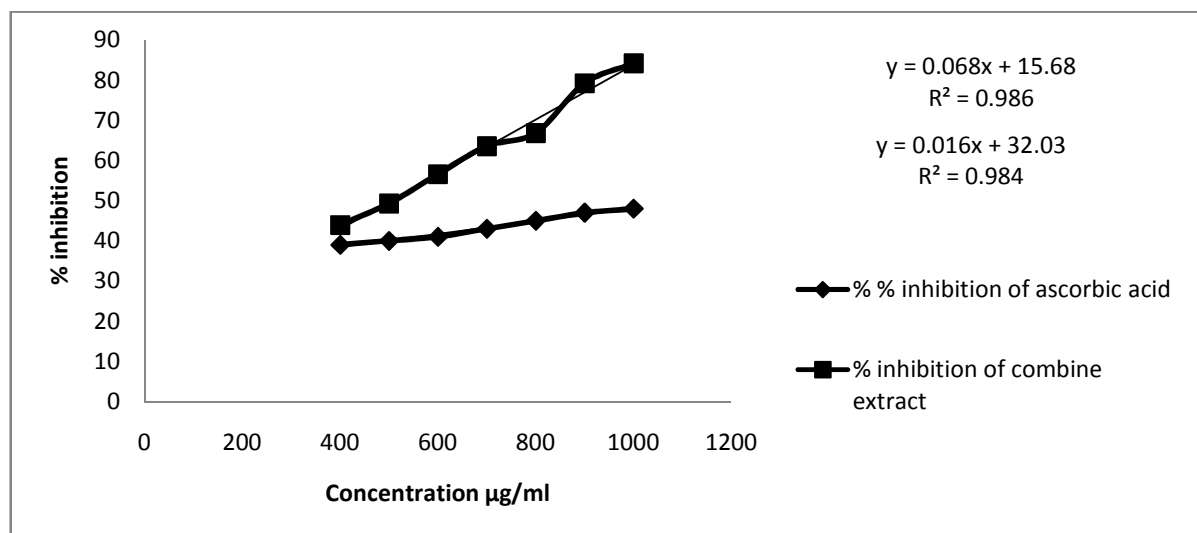


Figure II: Determination of IC₅₀ values for standard and combine extract from linear correlation between concentrations (µg/ml) verses percentage of scavenging of H₂O₂.

Table II: Total phenol and flavonoids contents, DPPH and H₂O₂ scavenging activities of Combine extract of *Azadirachta indica*, *Ocimum sactum*, *Aloe barbandesis*, *Tinospora cordifolia*.

| Sample | Total phenol content | Flavanoid content | DPPH radical scavenging, IC ₅₀ (µg/ ml) | H ₂ O ₂ radical scavenging, IC ₅₀ (µg/ ml) |
|-----------------|----------------------|-------------------|--|---|
| Combine extract | 13.75 mg/g | 16.14 mg/g | 514.1 µg/ ml. | 504.7 µg/ ml. |

IC₅₀ for Ascorbic acid was 498.1 µg/ml.

RESULTS

The percentage yield of *Azadirachta indica*, *Ocimum sactum*, *Aloe barbandesis*, *Tinospora cordifolia*, *Triticum aestivum* was found to be 7-17% w/w and the phytochemical screening of all extracts is depicted in the above table. Total phenol compounds, as determined by Folin Ciocalteu method, are reported as Gallic acid equivalents by reference to standard curve ($y = 0.0032x + 0.009$, $R^2 = 0.984$). The total flavonoid contents are reported as mg rutin equivalent/g of extract powder, by reference to standard curve ($y = 0.0007x + 0.049$, $R^2 = 0.989$) (table 2). It was noted that this combine extract possess higher total phenol and flavanoids. It was found that the radical-scavenging activity of extract increased with increasing concentration with IC₅₀ 514.1 µg/ ml. The extracts were also capable of scavenging hydrogen peroxide in a concentration-dependent manner with IC₅₀ 504.7 µg/ ml.

DISCUSSION

The presence of flavonoids and tannins in the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers [14]. Phenols and polyphenolic compounds, such as flavonoids, are derived from plant sources; possess significant antioxidant activities [15].

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples [16]. DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [17]. Usually, higher total phenol and flavonoids contents lead to better DPPH-scavenging activity [18].

Scavenging of H₂O₂ by extract may be attributed to their phenolics, which can donate electrons to H₂O₂, thus neutralizing it to water [19]. The ability of the extracts to effectively scavenge hydrogen peroxide, determined according to the method where they are compared with that of Gallic acid as standard.

In conclusion, this combine extract exhibited antioxidant activity in all the models studied. Further investigation of individual compounds, their in vivo antioxidant activities and in different antioxidant mechanisms are needed.

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