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In vitro study of antioxidant potential activity of *Terminalia arjuna*

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Abstract
Herbal medicinal plants are widely used as a therapeutic medicine for the treatment of wide range of various types of diseases all over the world. *Terminalia arjuna* is an important plant which is used as folk medicinal plant for the treatment of various complications. The present study was aimed to determine the antioxidant activity of the petroleum ether extract of the plant. The phytochemical analysis of *Terminalia arjuna* has indicated the presence of flavonoids and carotenoid. Petroleum ether extract of *Terminalia arjuna* leaves was evaluated for antioxidant property by DPPH scavenging assay method. The different concentration of the extract i.e. 50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml were showed 36.6%, 39.2%, 56.9% and 63.3% inhibition activities respectively, while BHT was used as standard which showed 79.4% inhibition activity. The above result suggested that *Terminalia arjuna* have some antioxidant potential activity. The statistical analysis showed significant result. Finally, we concluded that the antioxidant activity of the plant is may be due to the presence of various bioactive secondary metabolites.

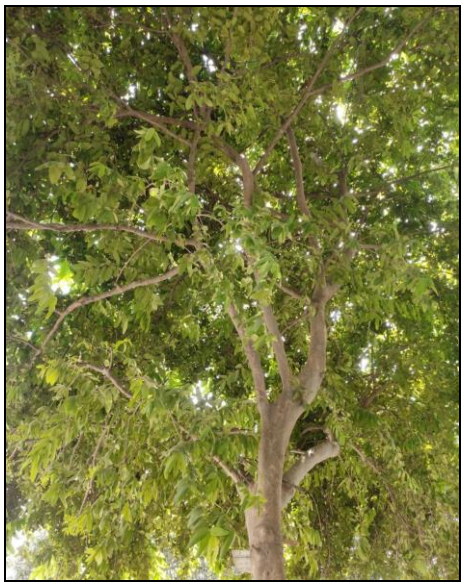
Keywords: *Terminalia arjuna*, antioxidant properties, petroleum ether extract, BHT

Introduction
numerous constant degenerative infections like malignant growth, diabetes, neurodegenerative ailment, atherosclerosis, cirrhosis, jungle fever and AIDS (Azizova *et al* 2002; Sian *et al* 2003; Quintero *et al* 2006; Nagler *et al* 2006) [3, 13, 10, 8]. Reactive oxygen species (ROS), including superoxide free radical, hydrogen peroxide, hydroxyl free radical and singlet oxygen assume a key job in the oxidative harm of these infections (Vertuani *et al* 2004) [19]. Cell reinforcement is an atom, which end the chain response by evacuating free extreme intermediates. Plants and creatures keep up complex arrangement of different kind of cell reinforcement; the regular plant based cancer prevention agents are assuming a significant job in the support of human wellbeing for as far back as three decades (Devasagayam *et al* 2004) [5]. There is an expanding enthusiasm for cell reinforcements, especially in those proposed to forestall the assumed harmful impacts of free radicals in the human body and to forestall the weakening of fats and different constituents of staples. In the two cases, there is an inclination for cell reinforcements from common as opposed to from manufactured sources (Abdalla and Roozen 1999) [1]. There is along these lines an equal increment in the utilization of techniques for evaluating the effectiveness of such substances as cell reinforcements (Sa'nchez-Moreno 2002) [11]. In this manner, the current investigation was embraced to assess and analyze the antioxidative exercises of various dissolvable concentrates of *T. arjuna* in various strategies.

Materials and Method
Plant materials
The plant materials were collected nearby the Dolphin (P.G) Institute of Biomedical and Natural Sciences, Manduwala, Dehradun, U.K, during the month of March. Collected plant materials were washed with tap water to remove mud and other undesirable material.

Methods
The collected plant Material was dried under shade after washing. The air-dried leaf & bark of *Terminalia arjuna*(Figure a, b) were crushed in to small pieces with the help of mortar and pestle then about 600gm of plant material was taken and extracted with petroleum ether by soxlet method.

The ethanolic extract was filtered by Buchner funnel and the filtrate was concentrated with rotatory evaporator at 40 ˚C. The concentrated extract was kept inside the refrigerator at 6-8 ^{0}C.



a). *Terminalia arjuna* plant

b). Bark

Biochemical Analysis

Test for Flavonoids:

a) NaOH Test: 100 µl Extract solutions were treated with few drops of sodium hydroxide solution, then the yellow color of the solution was the result. This becomes colorless on addition of dilute HCl which showed the presence of flavonoids.

b) Lead Acetate Test: In **t**o 1 ml extract solution, few drops of lead acetate solution was added which produced yellow precipitate that showed the presence of flavonoids.

Test for Alkaloids:

Plant extract was dissolved individually in dil HCl and filter, and then filtrate was treated with saturated picric acid which produced brown precipitate that indicated the presence of alkaloids.

Methodology of Anti oxidant activity by DPPH assay

The free radical scavenging activity of the plant extract was determined using the stable free radical's DPPH (1, 1-diphenyl-2-picrylhydrazyl) and butylated hydroxyl toluene (BHT) used as a positive control.Different concentration of the *Terminalia arjuna* extract were prepared in methanol. *viz.* 50 µg/ml, 100 µg/ml, 150 µg/ml and 200 µg/ml. The concentration of BHT was 20 mg/ml also prepared in methanol which was used as positive control. The solution of DPPH of concentration 0.1 mM /ml was prepared and kept in dark and then 1.0 ml of this solution was added to each tube containing 2ml of different concentration of the test sample prepared and also to the tube containing BHT. A blank was prepared by adding extract and methanol. The samples were then mixed well and incubated for 30min in dark, after which the absorbance was measured at 517nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated, using the following equation:

$$\% \text{ Activity} = \frac{\text{Acontrol} - \text{Atest}}{\text{Acontrol}} \times 100$$

(Where a control is the absorbance of the control (BHT) and A test is the absorbance of the test samples of the extract). Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPH-H and as consequence the absorbance decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Determination of Anti-oxidant activity by DPPH method

DPPH assay was performed as given below

Preparation of different concentration of *Terminalia arjuna* extract

Different concentration of the test sample was prepared. *viz.* 50 µg/ml, 100 µg/ml, 150 µg/ml and 200 µg/ml in methanol.

Preparation of standard BHT solution

BHT is a strong anti-oxidizing agent and is taken as standard. 20 mg/ml solution of BHT was prepared in methanol.

Preparation of DPPH

DPPH is a highly oxidizable compound. It oxidized in light, so DPPH is prepared in dark. Weigh accurately 20 mg DPPH and dissolved in solvent. Generally, methanol and for some cases Ethanol is used as a solvent for DPPH.

Preparation of test sample

2 ml of different concentration of *Terminalia arjuna* extract was mixed with 1 ml of DPPH solution in dark and incubated at 36^{0}C for 30-60 minutes.

Measurement of absorbance

After the completion of incubation, absorbance was taken with the help of U.V. Spectrophotometer at 517 nm.

Calculation

We calculated the % activity of individual concentration of the plant extract using the following formula: -

$$\% \, Activity = \frac{Abs.\,of\,controll - Abs.\,of individual concentration}{Abs.\,of\,controll} \times 100$$

Depolarization also gives rise to the deep violet colour, characterised by an absorption band in methanol solution centred at about 517 nm.

Result

Preliminary phytochemical analysis of *Terminalia arjuna* leaf extract:

Chemical Constituents	Seed extract
Flavonoids	+
Alkaloids/Carotenoids	+

(+ = present, - = absent)

Antioxidant activity of leaf extract

The antioxidant activity of the crude extracts was elucidated by the DPPH radical scavenging assay.

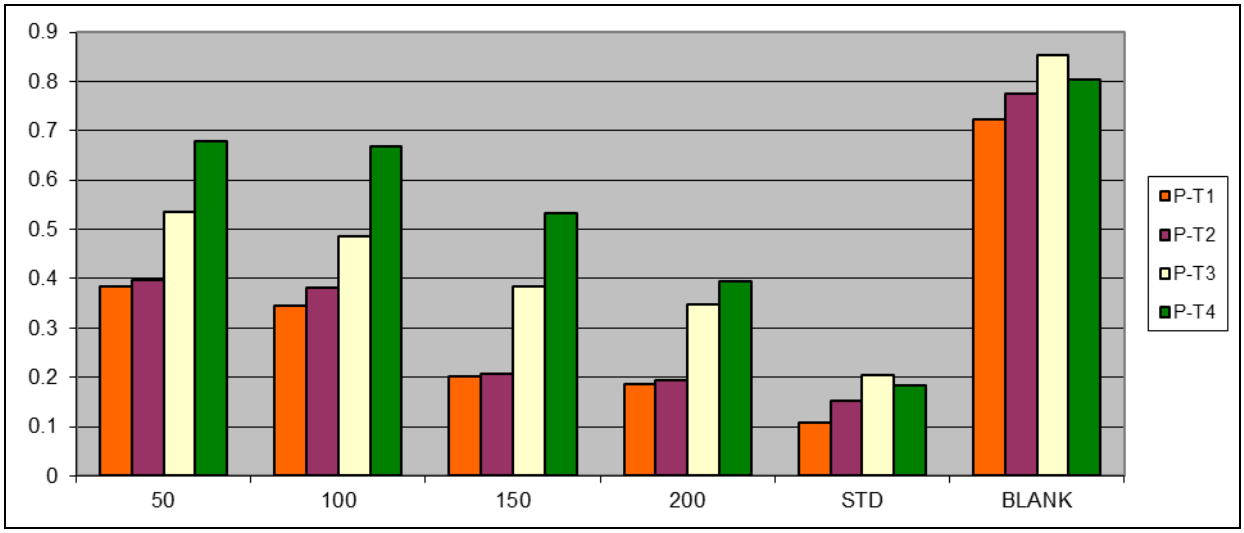
Determination of Free Radical Scavenging Activity

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). It is very convenient to follow the DPPH reactions and it has often been used to estimate the antiradical activity of the natural products. The decrease in DPPH absorbance in the presence of varying concentrations of extract has been monitored. The following results were obtained after carrying out the experiment.

Table 1: Absorbance obtained and the *I%* at different concentration of petroleum ether extract of *Terminalia arjuna*

Sl. No.	Samples (in µg/ml)	Absorbance at 517nm	$\frac{A_{control} - A_{test}}{A_{control}} \times 100$	I%
1	Methanol +ve control	0.788	------	------
2	BHT (standard)	0.162	$\frac{0.788 - 0.162}{0.788} \times 100$	79.4%
3	50 µg/ml (plant extract)	0.499	$\frac{0.788 - 0.499}{0.788} \times 100$	36.6%
4	100 µg/ml (plant extract)	0.479	$\frac{0.788 - 0.479}{0.788} \times 100$	39.2%
5	150 µg/ml (plant extract)	0.339	$\frac{0.788 - 0.339}{0.788} \times 100$	56.9%
6	200 µg/ml (plant extract)	0.289	$\frac{0.788 - 0.289}{0.788} \times 100$	63.3%

The above (table1) showed the values of the absorbance at 517 nm, for the different concentrations of the plant extract. It can be noticed that the extract at high concentrations showed significant decrease in the absorbance of DPPH radical (i.e. 200 µg/ml ($A_{0.289}$) <150 µg/ml ($A_{0.339}$) <100 µg/ml ($A_{0.479}$) <50 µg/ml ($A_{0.499}$) and the inhibition activity showed 36.6%, 39.2%, 56.9%, 63.3% respectively. The statistical analysis also showed the significant result. (Table2) A bar graph of the absorbance (vertical) obtained against the increase in concentration of the extract. The graph clearly showed the relationship between the absorbance with the increase of concentration. i.e. the absorbance decrease with increase in concentration.



Graph 1: Bar graph showing the decrease in absorbance at 517 nm after mixing petroleum ether solution of DPPH radical with different concentration of extract.

Discussion

Flavonoids and alkaloids are compounds found in fruits, vegetables, and certain beverages that have diverse beneficial biochemical and antioxidant effects. Their dietary intake is quite high compared to other dietary antioxidants like vitamins C and E. The antioxidant activity of flavonoids depends on their molecular structure, and structural characteristics of certain flavonoids found in hops and beer confer surprisingly potent antioxidant activity exceeding that of red wine, tea, or soy. Naturally antioxidant present in many

plants, foods and beverages offer health benefits in preventing various diseases by fighting cellular damage caused by free radicals in the body. The Presence of flavonoids, steroids and alkaloid in the plant extracts, indicates the plant having antioxidant activity.

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