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PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF *GARDENIA JASMINOIDES* ELLIS AND *DIOSPYROS MALABARICA* KOSTEL

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ABSTRACT

This paper describes the investigative report on phytochemical constituents and antioxidant activity of two plants, *Diospyros malabarica* and *Gardenia jasminoides* for their potential as therapeutics. Diseases like cancer, rheumatoid arthritis, liver diseases and atherosclerosis as well as in degenerative processes associated with ageing are the consequence of various metabolic activities in our body that results in the formation of the free radicals. Antioxidant compounds play an imperative role as a health defending factor and are defined as free radical scavengers. The present study revealed higher ascorbic acid content and total phenolic content in *D. malabarica* than that of *G. jasminoides*. Free radical scavenging activity was evaluated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The overall antioxidant activity of *D. malabarica* was more than that of *G. jasminoides*. All the methanolic extracts exhibited antioxidant activity significantly in both the plant extracts. The IC_{50} value of the methanolic extracts ranges from 8.68 ± 1.43 to 61.17±0.65 µg/ml. This report is thus suggestive of the potential of these two plants as therapeutic agent by virtue of their antioxidant activity.

KEYWORDS: ***Diospyros malabarica, Gardenia jasminoides,*** **antioxidant activity, total phenolic content, ascorbic acid, DPPH, Gallic acid.**

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INTRODUCTION

Production of certain free radicals in the human body such as superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals are the consequence of body's natural metabolic processes. This can trigger the onset of many diseases such as cancer, liver diseases, rheumatoid arthritis, and atherosclerosis as well as in degenerative processes associated with ageing[1]. However, inbuilt antioxidant systems such as superoxide dismutase (SOD), tissue glutathione (GSH) etc. protect the tissues from free radical attack[2]. Natural antioxidants such as vitamin C, E, carotenoids, phenolic compounds, etc. that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress exerted by the reactive oxygen species (ROS)[3] and are much more safer and cheap then the commercially available antioxidative products. It has been reported that the antioxidant activity of plant materials are well correlated with the content of their phenolic compounds[4,5] and ascorbic acid content. Since naturally available antioxidants are much more beneficial to the mankind, so identification of novel medicinal plants having antioxidative activity can hold a great future prospect in the field of medicine as this may lead to the development of new therapeutic drugs. *Diospyros malabarica* KOSTEL (family Ebenaceae) grows throughout India and other tropical regions of the world. The bark of *D. malabarica* KOSTEL has been reported to have high antioxidant activity and thus possess hepatoprotective efficacy and various other therapeutic applications[6, 7]. *Gardenia jasminoides* ELLIS is an evergreen flowering plant of the family Rubiaceae. It originated in Asia and is most commonly found in Vietnam, Southern China, Taiwan, Japan, Myanmar and India. The fruits of *Gardenia jasminoides* ELLIS are being used as herbal medicines and natural dyes in China since a long time. The fruits contain crocin, a member of carotenoid family, which is mainly responsible for their antioxidant property[8]. But there is no such report about the antioxidant activity of the leaf and the bark of *Gardenia jasminoides* ELLIS and the fruits of *Diospyros malabarica* KOSTEL. So the present study was subjected to evaluate the antioxidant activity of leaves and barks of *Gardenia jasminoides* ELLIS and the fruits of *Diospyros malabarica* KOSTEL.

MATERIALS AND METHODS

(i) ***Collection and preparation of plant materials***

The leaves and barks of *Gardenia jasminoides* ELLIS and the fruits of *Diospyros malabarica* KOSTEL were collected from the Gauhati University campus, Guwahati, Assam. The collected samples were washed thoroughly, sliced and oven dried at 60 ^{0}C until they were completely dried and get constant weight. The dried slices were then powdered and kept at 4 ^{0}C for further analysis. The plant powder was used directly for the preparation of the crude extract in different solvents as per necessity of the experiment. Chemical analysis was done on moisture free basis to estimate the total phenolic content, ascorbic acid and antioxidant activity of the samples.

(ii) Total Phenol Content Estimation

The total phenol content was determined by the Folin- Ciocalteau's method[9]. For the purpose 1 gram each of fruit, stem and leaf samples were ground with a pestle and mortar in 10 ml of 80% ethanol. The homogenates were centrifuged at 10000 rpm for 20 minutes and then the supernatant was evaporated to dryness. The residue of each extracted sample was dissolved in distilled water so as to make final concentration of the extract 1mg/ml[9]. 200 µl of each of these plant extract were taken and volume made up to 2 ml. 0.3 ml of Folin-Ciocalteau reagent was added. After 5 minutes, 0.8 ml of 20% Na_2CO_3 was added and the final volume was made 5 ml. Absorbance was taken by UV-Vis Spectrophotometer at 765 nm after 30 minutes incubation. The amount of phenol content was determined using Gallic acid as standard. Results were expressed as µg/mg (Gallic acid equivalent/dry weight).

(iii)Ascorbic Acid Content Estimation

The amount of ascorbic acid present in the samples was calculated by extracting the

sample in 4% oxalic acid and titrating the extract against the 2, 6-dichloro phenol indophenol dye until the end point where pink colour appears that persist for a few minutes[10]. The amount of dye consumed is equivalent to the amount of ascorbic acid present in the samples. Standard ascorbic acid solution is used as the reference and the calculation is done by the following formula:

$$\text{Amount of ascorbic acid}\left(\frac{\text{mg}}{100\text{g}}\right)\text{sample} = \frac{0.5 \times V_1\ \text{ml} \times 100\ \text{ml}}{V_2\ \text{ml} \times 5\text{ml} \times \text{weight of the sample}} \times 100$$

Where, V_1= volume of oxalic acid, V_2= volume of the sample.

(iv) Antioxidant activity estimation

The antioxidant activities of the plant extracts along with standard were assessed on the basis of the radical scavenging effect of stable DPPH[11]. A solution of DPPH of concentration 0.2 mM was prepared in 70% methanol and kept overnight. Stock solution (1 mg/ml) of the extract was prepared in 70% methanol. Various concentrations of the extracts *viz.*10, 20, 50,100,150,200,300,400 and 500 µl were taken in different test tubes and the volume was made up to 1000 µl. 1 ml DPPH was added to each solution and kept at dark for 30 minutes. Ascorbic acid and Gallic acid were taken as standards. Optical density of these samples was measured at 517 nm along with blank where 1 ml methanol with 1 ml DPPH solution was taken. The activities of the samples are measured in terms of percent inhibition (IC_{50}) and calculated by the following formulae:

$$\text{Percent (\%) inhibition of DPPH activity} = \frac{A - B}{A} \times 100$$

Where, A = Optical density of the blank, B = Optical density of the sample

(v) Statistical Analysis

The data were subjected to statistical analysis. All the assays were recorded in triplicates and the values were expressed as mean ± S.D. IC_{50} value was calculated by plotting a graph with percent inhibition on y-axis and concentration on x-axis**.**

RESULTS AND DISCUSSION

The phytochemical analysis was done for total ascorbic acid content and total phenol content in both the extracts of *Diospyros malabarica* and *Gardenia jasminoides* .The antioxidant activity was measured by using DPPH assay. In *Diospyros malabarica,* the total phenol content and ascorbic acid content were found to be higher as compared to *Gardenia jasminoides* (Table 1).

Table 1
Phytochemical analysis of Diospyros malabarica and Gardenia jasminoides.*

Sample	Ascorbic acid (mg/100gm)	Total phenolic content (µgGAE/mg)	Inhibition Concentration (IC_{50}) (µg/ml)
Gardenia jasminoides (leaf)	34.17±0.56	96.85±1.80	48.97±0.85
Gardenia jasminoides (bark)	26.23±0.96	89.43±0.33	61.17±0.65
Diospyros malabarica (fruit)	55.57±0.75	223.5±0.26	08.68±1.43

Values represented in the table are mean ± S.D of three replicates.

The antioxidant activity of *Gardenia jasminoides* is very promising with respect to its usage as a medicinal plant as it had an inhibition concentration (IC_{50}) of 48.97 ± 0.85 µg/ml and 61.17 ± 0.65 µg/ml in leaves and bark respectively (Fig.1 and Fig.2). The antioxidant activity of fruit of

Diospyros malabarica was found to be significantly high (Fig. 3) and it had an inhibition concentration of IC_{50}= 08.68 ± 1.43 µg/ml (Table 1).

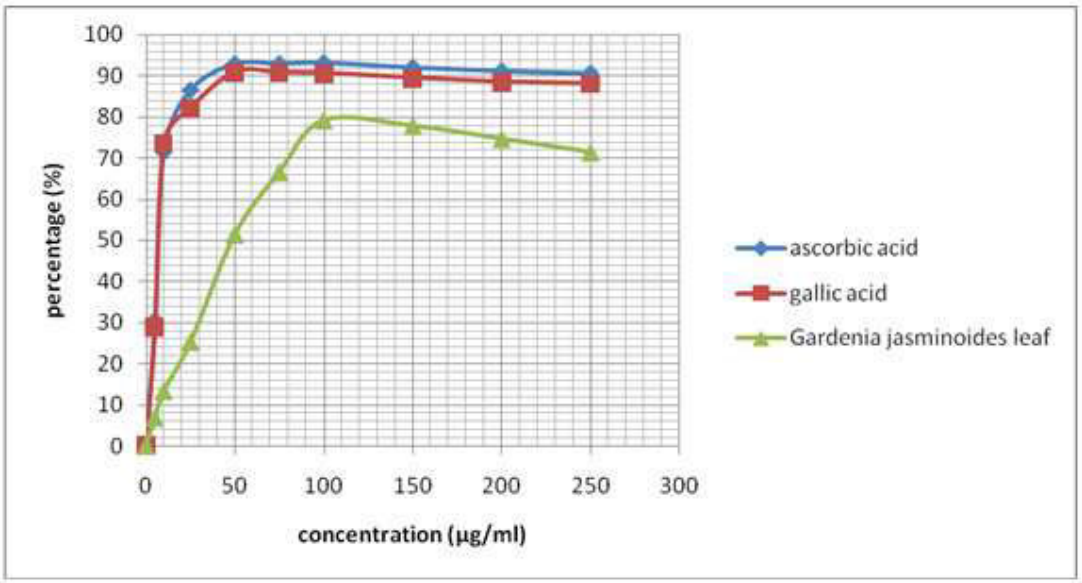

Figure 1
DPPH free radical scavenging activity of methanolic leaf extracts of Gardenia jasminoides ELLIS 517 nm.

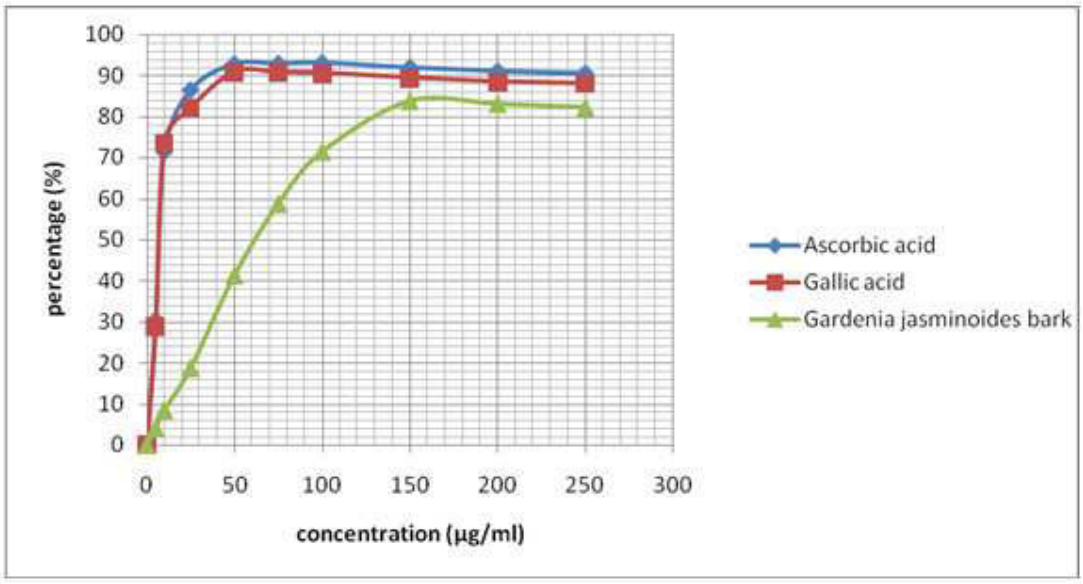

Figure 2
DPPH free radical scavenging activity of methanolic bark extract of Gardenia jasminoides ELLIS at 517 nm.

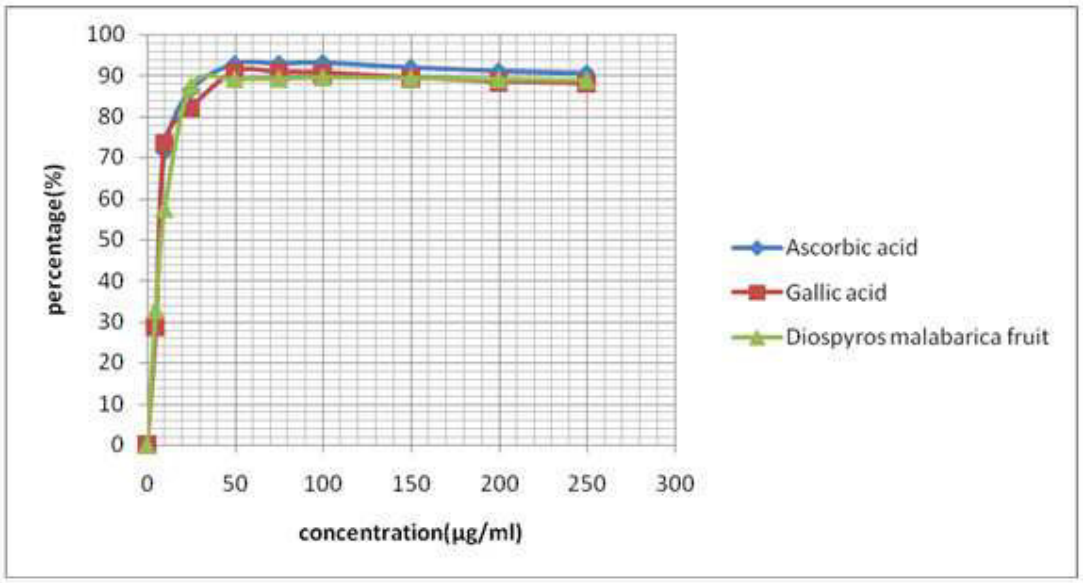

Figure 3
DPPH free radical scavenging activity of methanolic fruit extract of Diospyros malabarica KOSTEL at 517 nm.

The most common antioxidants present in herbs and fruits are vitamins C and E, carotenoids, flavonoids and thiol (SH) compounds, etc. There were several reports which revealed that the contribution of phenolic compounds to antioxidant activity was much greater than those of vitamin C and carotenoids[12,13,14]. The present investigation also unveiled the role of phenolic components as major source of antioxidant activity in *Diospyros malabarica* and *Gardenia jasminoides*. The protection provided by medicinal plants against oxidative damage to body tissues has been attributed to the fact that these foods may provide an optimal mix of phytochemicals, such as natural antioxidants and other bioactive compounds. Moreover there is a scope that these plants can be used as a therapeutic agent against various liver diseases as they may possess hepatoprotective activity by virtue of its antioxidative potential.

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