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Free radical scavenging activity and reducing power of *Gnidia glauca* (Fresen.) Gilg

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ABSTRACT

The present study describes the free radical scavenging activity and reducing power of methanolic leaf extract of *Gnidia glauca* (Fresen.) Gilg.using ABTS [2, 2' azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)], FRAP (Ferric reducing antioxidant power) and nitric oxide scavenging assays. Total antioxidants were determined by phosphomolybdenum method. The results have indicated that the total phenolic content of the extract was 203.3 GAE/g and showed significant antioxidant activity with an IC_{50} value of 16.3µg/mL in ABTS and 360.8µg/mL in nitric oxide scavenging assays respectively. A FRAP value of 993.7 µm TE/mg of leaf extract was recorded at 30 minutes. The total antioxidant activity was 142.5 mg AAE/g of extract. The results strongly establish the significant antioxidant property of *G. glauca* owing to its high phenolic content.

INTRODUCTION

Plants represent a major source of biologically active molecules and only a small fraction of plants with medicinal activity have been explored. The rich traditional knowledge of mega biodiversity nations like India and China on medicinal plants and health care has been the basis of research and development by pharmaceutical companies to develop novel drugs for major ailments. The chain reaction set up by the unstable free radicals is effectively terminated by antioxidants. Various free radicals are known to cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's disease, mongolism, ageing process and perhaps dementias (Polterait, 1990). These free radicals exert oxidative stress on the body cells, rendering each cell susceptible to 10 000 oxidative hits per second (Lata and Ahuja, 2003). The free radicals are highly reactive species capable of damaging DNA, proteins and lipids in the cells. Dietary antioxidants have an important role as nutraceuticals due to their ability to protect the body from cascading ill effects of free radicals (Vijaykumar *et al.*, 2013). At present, most of the antioxidants used are manufactured synthetically which are known to have adverse side effects *in vivo* (Ramamoorthy and Bono, 2007). Studies have reported that the most widely used antioxidants like butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT) get accumulated in the body and cause liver damage beside acting as carcinogens (Jiangning*et al.*, 2005). Further, most of these synthetic antioxidants show moderate antioxidant activity, low solubility (Barlow, 1990; Branen, 1975) and therefore natural antioxidants from plant sources are considered as viable alternatives. The extracts of medicinal plants and natural products have become a great source of antioxidant and anti-ageing compounds (Sumazian, 2010). *Gnidia glauca* (Fresen.) Gilg (Thymelaeaceae), is reported to be used widely in folkloric medicine. The genus *Gnidia* possesses a variety of traditional phytomedicinal uses and agrochemical applications. It is used in traditional African medicine to treat cancer, sore throat, abdominal pain, wounds, burns and snake bites. It is also used as molluscicidal, insecticidal, piscicidal, (Borris and Cordell, 1984; Franke *et al.*, 2002; Amarajeewa *et al.*, 2007) homicidal agent and as a source of arrow poison by tribals.Leaves of *Gnidia glauca* have been used for the treatment of contusions, swellings, back ache and joint aches (Kareru *et al.*, 2007). The species *Gnidia glauca* has been studied for its antihelminthic, antifungal and antidiabetic properties (Nethravathi *et al.*, 2010; Kharde *et al.*, 2010; Ghosh *et al.*, 2012). Though the free radical scavenging ability of the plant extracts has been reported against DPPH radical, no systematic study has been conducted to assess the antioxidant potential of *Gnidia glauca* which forms the basis of this research work.

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MATERIALS AND METHODS

Plant material

Fresh leaves of *Gnidia glauca* were collected during August 2012 – February 2013 from Kuppali and Jaipura regions of Shringeri, Western Ghats, India. The specimen was identified using Flora of Coorg (Keshavamurthy and Yoganarasimhan, 1990) and further authenticated by the National Ayurveda Dietetics Research Institute (Central Council for Research in Ayurveda and Siddha) with reference number Drug authentication/ SMPU/ NADRI/ BNG/2012-13/1007.

Source of chemicals

All the chemicals used in this study were procured from Sigma, Himedia, SD Fine and Merck and all were analytical grade.

Preparation of plant extract

Fresh leaves were washed repeatedly to remove adhered dust particles, blotted, shade dried, powdered using an electric blender and stored in air tight container. About 300g of the leaf powder was subjected to methanolic extraction under reflux for 1 hour. The procedure was repeated thrice until complete extraction was achieved.

Estimation of total phenolic content

The total phenolic content of the methanolic leaf extract was determined by Folin–Ciocalteau reagent (Singelton and Rossi, 1965). Briefly, 40 µl of the methanol solution of the extract (1 mg/ml), 200 µl of Folin–Ciocalteau reagent was mixed with 2.4 ml of distilled water and the contents were mixed thoroughly. After 1 min, 600 µl of sodium carbonate (20% Na_2CO_3) was added and the volume was made up to 4.0 ml with distilled water. After 2 h of incubation at room temperature, the reduction of the Folin–Ciocalteau reagent by phenolic compounds under alkaline conditions resulted in the development of a blue colour, the absorbance was measured at 765 nm and compared to a gallic acid calibration curve. Results were expressed as mg of gallic acid equivalents (GAE) /g of extract.

ABTS assay

The 2, 2' azinobis 3-ethylbenzothiazoline-6-sulphonate (ABTS) assay was performed as per Auddy *et al.,* (2003) where in radical cations were produced by reacting ABTS with ammonium persulphate and incubating the mixture at room temperature in dark for 16 hours. The total reaction volume contained 20µL of 10mM PBS pH 7.4, test solutions of various concentrations and 230µL of ABTS radical solution (0.238mM). The reagents were mixed and the absorbance was immediately read at 734nm using blank run without test sample.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to Benzie *et al.,* (1996) with slight modifications suggested by Firuzi and Lancana (2005). FRAP reagent was prepared and kept at 37^{o}C for 5-10 minutes prior to use. In a 96 well plate, 175µL of FRAP reagent was added and the plate was pre read at 593 nm. To each well, 25 µL of sample solution was added and contents were mixed well using a pipette. Absorbance was measured at 593 nm at 4, 15, 30 and 60 minutes, using methanol as blank. Change in absorbance at different time intervals was then translated into a FRAP value using a standard trolox curve.

Total antioxidant activity

The total antioxidant activity of methanolic leaf extract was determined by using phosphomolybdenum (Prieto *et al.,* 1999). An aliquot of 0.4 mL of methanolic leaf (1 mg/mL) was mixed with 4 mL of the phosphomolybdenum reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in water bath at 95º C for 90 minutes, cooled and the absorbance was read at 695 nm. The antioxidant activity of the sample was determined using a standard curve of ascorbic acid. The reducing capacity of the extract was expressed as mg of ascorbic acid equivalents (AAE)/g of extract.

Nitric oxide scavenging assay

About 1 mg of the methanolic leaf extract was dissolved in 20µL of methanol and the volume was made up to 1 mL with distilled water. Further dilutions were done to final concentrations of 25, 50, 100, 250, and 500µg/mL using vehicle buffer.

Nitric oxide was generated from sodium nitroprusside and was measured by the Griess reagent. The reaction mixture contained 50 µL of 10 mM sodium nitroprusside**,** 50 µL of distilled water and test solution of various concentrations. The reaction mixture was pre-incubated at room temperature for 15 minutes in the presence of light, followed by the addition of 125 µLGriess reagent and incubated for 10 minutes at room temperature. The absorbance was measured at 546 nm (Sreejayan *et al.,* 1997).

RESULTS AND DISCUSSION

Total phenolic content

The mean phenolic content of the methanolic leaf extract was 203.3 mg GAE/g of extract. This indicates that *Gnidia glauca*is significantly rich in phenolic contents. Presence of flavonoid as a major phytoconstituent has been reported in *Gnidia glauca* (Nethravathi *et al.,* 2009). It is well established that flavonoids containing hydroxyl functional groups contribute to the antioxidant potential of the plant extracts (Das and Pereira, 1990; Younes, 1981).

Hakkim et al., (2008) reported lower total phenolic contents of 168.2 mg and 123.1 mg GAE/g respectively in *Ocimum gratissimum* and *Ocimum americanum.* This indicates that *Gnidia glauca*is significantly rich in phenolic contents. Phenolic compounds such as phenolic acids, polyphenols and flavonoids are commonly found in plants and have been reported to have multiple biological effects, including antioxidant activity (Brown and Rice-Evans, 1998). A strong correlation exists

between phenolic content and antioxidant activity of the plant extract, a property attributed to the free radical terminating potential of phenolic compounds (Shahidi and Wanasundara, 1992).

The presence of high phenolic content provides a direct evidence for the significant free radical scavenging potential of *Gnidia glauca*.

ABTS assay

ABTS assay is based on the scavenging of light by [2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radicals. An antioxidant shows a marked ability to donate a hydrogen atom which would consequently quench the stable free radical. Although ABTS assay is not biologically relevant, the assay is performed as a preliminary study to evaluate the radical scavenging abilities.

In the present study, the methanolic leaf extract of *Gnidia glauca* showed a high ABTS radical inhibition of 79.5% at a concentration of 40 µg/mL. There was a steady increase in the inhibition of the radicals with concomitant increase in concentration of the extract establishing dose dependence of the extract in scavenging ABTS radicals (Fig. 1). The IC_{50} value of leaf extract was 16.3µg/mL as against the standard Gallic acid (1.7µg/mL). Rasappan *et al.*, reported a higher IC_{50} of 85.4 µg/mL in *Psidium guajava*. Several species belonging to Thymelaeaceae also have been reported to possess significant antioxidant properties. ABTS scavenging activity of 92.57% at a concentration of 5 mg/mL has been reported in *Daphne kiusiana* (Jin *et al.*, 2011). In the present study, a lower IC_{50} value substantiates higher free radical scavenging properties of *Gnidia glauca*.

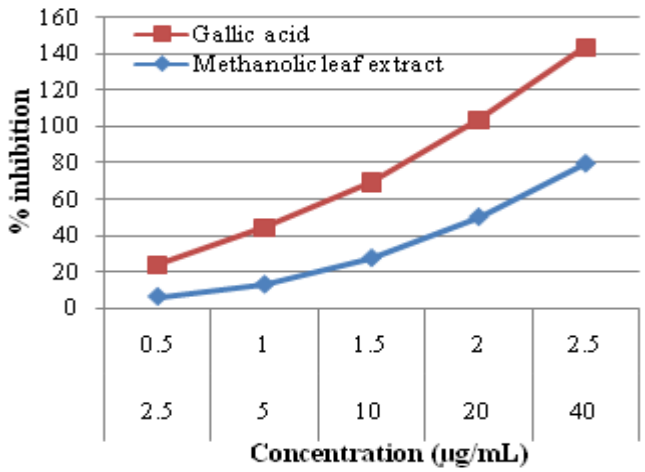

Fig. 1: Percentage inhibition of ABTS radicals by methanolic leaf extract of *G. glauca*.

FRAP assay

The results expressed as FRAP values at different time intervals, showed 596.8 at 4th minute and steadily followed an increasing trend to 993.7 at 30th minute further reaching 1116.8 at 60th minute (Fig. 2). The methanolic leaf extract of *G. glauca* has a significant ability to bring about reduction of ferric ions in a time dependent manner. At a concentration of 5 mg/mL, *Daphne kiusiana* has been reported to possess a reducing power of 7.2% (Jin *et al*., 2011).

The reducing power of a compound is related to its electron transfer ability. Reducing power is widely used to evaluate the antioxidant activity of polyphenols, which is associated with the presence of reductones, which exerts antioxidant activity by breaking the free radical chain by donating a hydrogen atom (Duan and Jiang, 2007). FRAP value serves as a measure of Fe (II) TPTZ reducing power of the extract.

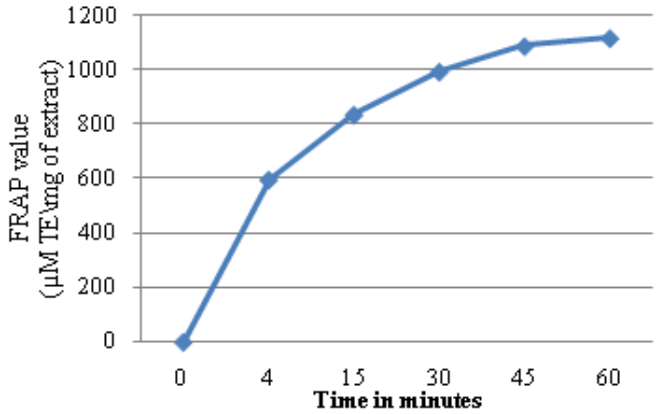

Fig. 2: FRAP value of methanolic leaf extract of *G. glauca* at different time intervals.

Total antioxidant activity

The phosphomolybdate method has been used routinely to evaluate the total antioxidant capacity of plant extracts (Prieto *et al*., 1999; Prasad *et al.*, 2009), for evaluating antioxidants such as ascorbic acid, some phenolics, α-tocopherol and carotenoids (Prieto *et al*., 1999). In the presence of methanolic leaf extract, Mo (VI) is reduced to Mo (V) and forms a green colored phosphomolybdenum V complex.

In the present study, the total antioxidant activity of methanolic leaf extract was 142.5mg AAE/g of extract. It showed a higher Mo (VI) reducing ability when compared to *Daphne cneorum*which has been reported to have lesser total antioxidant capacity with 70.55 mg AAE/g of extract (Manojlovic *et al*., 2012).

Nitric oxide scavenging assay

Nitric oxide is regarded as an important mediator of acute and chronic inflammation, which can easily react with superoxide anion to form peroxynitrite (ONOO-), a potent oxidizing molecule capable of eliciting lipid peroxidation and cellular damage (Beckman *et al*., 1990; Radi *et al.*, 1991; Rubbo *et al.*, 1994). In all, nitric oxide has the ability to exert multiple cytotoxic effects. Any plant with a considerable ability to terminate nitric oxide radicals *in vitro*, would prove to be an important source of natural antioxidant lead molecules. The methanolic leaf extract was tested for its nitric oxide scavenging potential. The inhibition percentage was evaluated for each test concentration and it ranged between 11.2 and 46.7% for leaf

extractand between 21.9 and 80.3% for the standard curcuminoid (Fig. 3). The IC_{50} value was 360.8µg/mL for the extractas against 24.6µg/mL for curcuminoid.

The methanolic leaf extract showed a significant nitric oxide scavenging ability which was higher when compared to that of *Chromolaena odorata* with an $IC_{50}$of 380 µg/mL (Alisi *et al.*, 2008). However, *Myena laxiflora* has been reported to possess a higher nitric oxide scavenging ability with an IC_{50} of 104 µg/mL (Ganesh *et al*., 2010). Therefore it is evident that *Gnidia glauca*possesses higher nitric oxide scavenging activity indicating its significant antioxidant potential.

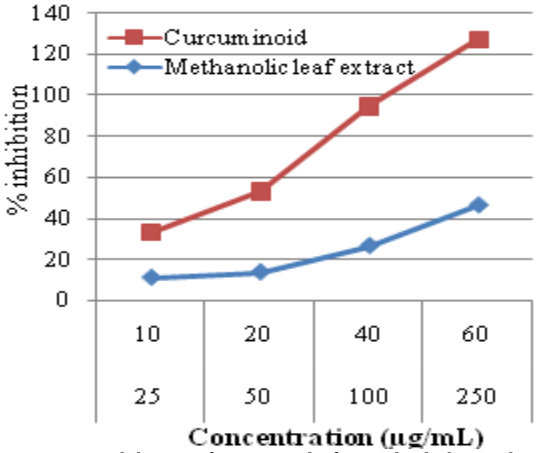

Fig. 3: Percentage inhibition of nitric oxide free radicals by methanolic leaf extract of *G. glauca.*

CONCLUSION

The test plant *Gnidia glauca* appears to be a promising source of bioactive compounds that would elicit interesting antioxidant effects in animal systems. The plant has significant antioxidant activity which is attributed to its high phenolic content. Therefore *Gnidia glauca* is regarded as excellent source for bioactive compounds that can be further developed into drugs to combat oxidative stress.

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