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In-vitro FREE RADICAL SCAVENGING ACTIVITY OF DIFFERENT PROPORTIONS OF *Glycyrrhiza glabra* AND *Tinospora Cordifolia*

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Abstract: The present study was carried out to evaluated the *in-vitro* antioxidant properties of different extracts of *Tinospora cordifolia* stem and of *Glycyrrhiza glabra* root alone and its combinations by DPPH (1, 1-Diphenyl-2-picrylhydrazyl) method. Chloroform and methanol extracts of both plants were prepared by soxhlet extraction and used to evaluated antioxidant properties. The free radical scavenging activity of different concentrations of different extracts of both plants alone and in combinations 2:1 and 1:2was evaluated. The extracts showed significant antioxidant activities compared to the ascorbic acid in a dose dependent manner.IC_{50} (half maximal inhibitory concentration value) value was also determined. The IC_{50}value of the methanolic extract of *T. cordifolia* was found lower (4.5µg/ml) than *G. glabra* (5.4µg/ml), whereas the value of $IC_{50}$of chloroform extract of *G. glabra* was found lower IC_{50} value than *T. cordifolia.* The IC_{50} of combination of *G. glabra* and *T. cordifolia* methanol extract in ratios of 2:1 and 1: 2 were found similar (3.8 µg/ml). Whereas the IC_{50}values of chloroform extract at both plants in ratio of 2:1 and 1:2 were 6.5µg/ml and 8.5µg/ml, respectively. It was conclude that compounds responsible for antioxidants activity in above both plants may be better extracted with methanol, further development of herbal formulation & its validation has been proposed.

Keywords: *T. cordifolia, Glycyrrhiza glabra,* Antioxidant, DPPH (1, 1-Diphenyl-2-picrylhydrazyl), Herbal formulation, Free radical scavenging

Abreviation: GGM, *G. glabra* methanol; GGC, *G. glabra* chloroform; TCM, *T. cordifolia* methanol; TCC, *T. cordifolia* chloroform; GGTC, *G. glabra* & *T. cordifolia*; C, Chloroform; M, Methanol.

INTRODUCTION

There are many biological processes occur in the animal body. Due to this biological oxidation process many Free radicals or reactive oxygen species (ROS) are formed. Free radicals includes hydroxyl radical, super oxide anion radical, hydrogen peroxide which can cause damage to the body due to its overproduction and contribute to oxidative stress (Diplock, 1994; Thomson, 1995). There are various chronic disorders like cancer, coronary

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artery disease, hypertension, diabetes etc (Lee *et al*., 2000) may occur due to oxidative damage of proteins, DNA and lipid. (Kris*et al*., 2002; Di Malteo and Esposito, 2003 and Behera *et al*., 2006).Free radicals are derived from two sources: endogenous sources, e.g., nutrient metabolism, ageing process etc. and exogenous sources, e.g., tobacco smoke, ionizing radiations, air pollution, organic solvents, pesticides etc. In body there is endogenous defense mechanism such as catalase, superoxide dismutase and peroxidase-glutathione system by which most of the free radical is scavenged (Rice-Evans and Bourdan, 1993). But these endogenous mechanism may not be completely able to scavenge the all free radicals therefore exogenous anti-oxidants from natural sources are required in the body.

From natural sources medicinal plants are the major source of exogenous anti-oxidants, so now a-days plants are one of the main sources of new pharmaceuticals and health care products. The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents (Ivanova *et al*., 2005). There is also use of synthetic anti-oxidants, such as butylated hydroxylanisole (BHA) and butylated hydroxytoluene (BHT) but it has limited use in foods, so in recent time scientist and researchers have search for natural anti-oxidants. The researchers have focused on natural antioxidants and numerous crude extracts and pure natural compounds have been recognized to have beneficial effects against free radicals in biological systems as anti-oxidants (Reena *et al*., 2004; Hazra *et al*., 2008 and Demiray*et al*., 2009).

T. cordifolia (Miers.) is distributed throughout tropical Indian subcontinent and China. It is a widely used shrub in folk and Ayurveda belonging to the family menispermaceae. It is evaluated that *T. cordifolia* have anti-spasmodic, anti-inflammatory, anti-allergic, anti-diabetic, antioxidant properties (Singh *et al*., 2003).

G. glabra is another medicinal plant which has been used by human beings for at least 4000 years. Roots of have demulcent, antacid, anti-ulcer (Ambawade *et al*., 2002) anti-inflammatory, expectorant, tonic, diuretic, laxative and sedative properties (Hikino., 1985). They also possess antipyretic (Lata *et al*., 1999), antimicrobial, anti-oxidant (Shrama *et al*., 2013), and anxiolytic (Ambawade *et al*., 1950) activities.

The objective of the present study was to evaluate the *in-vitro* antioxidant properties of different extracts of *T. cordifolia* stem and of *G. glabra* root alone and its proportions by DPPH (1, 1-Diphenyl-2-picrylhydrazyl) method.

MATERIALS AND METHODS

Collection of plant material: *T. cordifolia* stem powder and *G. glabra* root were purchased from reliable local suppliers.

Preparation of extract: Extract of both the plants was prepared by soxhlet extraction method. About 30 gm of powdered material was uniformly packed into a thimble and run in soxhlet extractor. It was exhaustible extracted with chloroform and methanol for 72 hours. After that extracts were filtered with the help of filter paper (Whatman No. 1) and solvent was evaporated in a rotary evaporator to get the syrupy consistency, then after the extract was kept in refrigerator at 4 ℃ to determine antioxidant activity.

Antioxidant activity: The scavenging activity of *T. cordifolia* stem and *G. glabra* root extracts was determined using DPPH assay (Senguttuvan *et al*., 2014).This method depends on the reduction of purple DPPH to a yellow colored solution. The determination of the disappearance of free radicals was done using a spectrophotometer. The remaining DPPH which showed maximum absorption at 517nm was measured. Each plant extract sample's stock solution (1mg/ml) was prepared and then combination of these two plant extract stock solution in 2:1 and 1:2 ratio were also prepared. One ml of a 100 µMDPPH solution was added to 3 ml of sample solution of different concentrations (5, 10, 15, 20µg/ml). These are test solutions. Ascorbic acid was used as positive control and prepared in the same manner as above. The absorbance values were measured at 517 nm and converted into the percentage antioxidant activity using the following equation:

$$\%\ Inhibition = \frac{Absorbance\ of\ Control - Absorbance\ of\ Test}{Absorbance\ of\ Control} \times 100$$

The entire assay were performed in triplicates and repeated three times at different time points and the absorbance was presented as mean ±SE.

RESULTS AND DISCUSSION

In study antioxidant activities of *T. cordifolia* stem powder and *G. glabra* root powder alone with combination of both extract of methanol and chloroform were investigated by in vitro DPPH method use. Both plants having antioxidant activities in methanol and chloroform extract but combination of both plants extract whose produce more effect than individual extract.

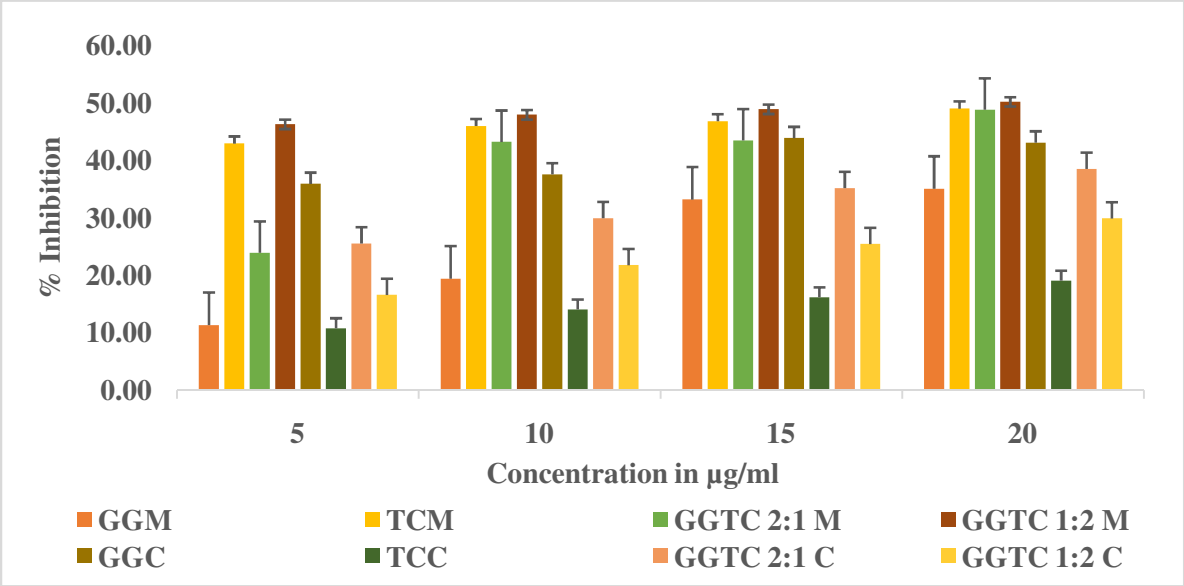

Fig. 1 DPPH scavenging activity of various extracts of *T. cordifolia and G. glabra*

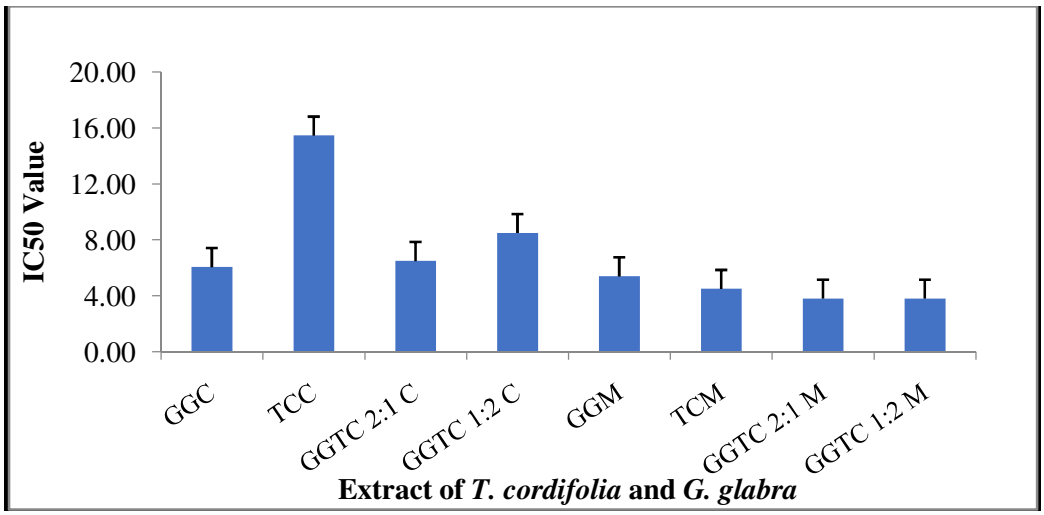

Fig.2 IC_{50} values of extracts of *T. cordifolia* and *G. glabra* in different proportions

Result showed that, the IC_{50} value of the methanolic extract of *T. cordifolia* was found lower (4.5 µg/ml) than *G. glabra* (5.4 µg/ml). The IC_{50} of combination of *G. glabra* and *T. cordifolia* methanolic extract in ratios of 2:1 and 1: 2 were found similar (3.8 µg/ml). Total phenolic content was found higher in methanol extract than chloroform extract of *T. cordifolia,* whereas phenolic content of methanol extracts was higher than chloroform extracts of *G. glabra.* Results revealed that, in the ambient conditions free radicals from DPPH were inhibited by both extracts as well combinations of these two. IC_{50} value for the *T. cordifolia* chloroform extract was found higher (15.8 µg/ml), this may be due to presence

of more amounts of non-polar compounds like Phytosterols and lipids (Fig.2). But it was very interesting to report that, when chloroform extract of *T. cordifolia* combined with *G. glabra*, the IC_{50}value was found to be almost half of the former one i.e. 8 µg/ml in the ratio of 2:1 (Fig.2). This may be due to the synergistic effect of phytochemicals present in both the plants i.e. flavonoids and glycosides of *T. cordifolia* and triterpenoid acid in *G. glabra*. One more interesting finding observed was in case of methanol extracts of both plants shown lowest IC_{50} value (3.8µg/ml).

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