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IN VITRO ANTIOXIDANT ACTIVITY OF *GLINUS OPPOSITIFOLIUS, ERYTHRINA STRICTA* AND *TRIGONELLA FOENUM - GRAECUM*

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ABSTRACT

Aim of the study is to carry out *in Vitro* Antioxidant Activity of *Glinus Oppositifolius, Erythrina Stricta* And *Trigonella Foenum – Graecum.* Very good activity of ethanolic extract of *Glinus oppositifolius, Erythrina stricta* and *Trigonella foenum-graecum* is probably due to the presence of substance with an available hydroxyl groups. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

Key Words: Antioxidant Activity, *Glinus Oppositifolius, Erythrina Stricta, Trigonella Foenum*

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INTRODUCTION

Oxidation of a compound can be defined as the removal of an electropositive atom, radical or electron, or the addition of an electronegative atom or radical. Oxidation is essentially a burning process, which generally uses oxygen as a component during the process, even more chemically reactive species are generated that can corrode (oxidise) any material nearby. Oxygen free radicals or oxidants are potentially problematic because they are more reactive than ground state molecular oxygen. Oxygen is present in the atmosphere as a stable triplet biradical ($^3O_2$) in the ground state and vital component for the survival of the human. Once inhaled it undergoes a gradual reduction process and ultimately gets metabolised into water. In these process, reactive intermediates like superoxide anion radicals ($O_2^{\bullet}$), hydroxyl radicals ($OH^{\bullet}$.), hydroperoxy radicals ($OHO^{\bullet}$) are all free radicals and are very reactive oxidants. Hydroxyl radical ($OH^{\bullet}$) is the most reactive and is capable of causing more damage to biological system. Hydrogen peroxide (H_2O_2) though not a radical is a reactive oxidant by itself as well as producing the $OH^{\bullet}$ radicals. These are collectively called as reactive oxygen species. A free radical may be defined as a molecule containing one or more unpaired electrons in its outermost orbital and are capable of independent existence. It is usually represented by the superscript ($R^{\bullet}$) some radical e.g. the hydrogen radical ($H^{\bullet}$), which contains one proton and one electron (unpaired) is the simplest free radical. Generally free radicals attack the nearest stable molecule "stealing" its electron. When the attacked molecule loses its electron it becomes a free radical. This leads to the beginning of a chain reaction, once the process is started, it can cascade finally resulting in the disruption of a living cell. Free radicals occur continuously in all cells as part of normal function. The free radicals are formed when oxygen interacts with certain molecules, once formed these highly reactive radicals can start a chain reaction

and cell may function poorly or die. A free radical reaction may be terminated by a reaction between two free radicals or by neutralization by substances such as the antioxidants. The term oxidative stress is defined as "a disturbance in the pro-oxidant antioxidant balance in favour of the former, leading to potential damage". For a disturbance in this balance to occur, it follows that one or both of the following scenarios must be present. Reduction in anti-oxidant can be brought about by three main mechanisms. Malnutrition may lead to inadequate intake of essential antioxidant nutrient. This can be seen in cases of neurodegeneration in patients with faulty fat absorption leading to vitamin E deficiency. Many drugs are conjugated with glutathione (GSH) in preparation for their excretion from the body leading to reduced GSH levels. Genetic mutations may adversely effect antioxidant systems leading to reduced antioxidant action. Increase in reactive species are thought to be the most common causes of oxidative stress in the human body. Increased oxygen concentrations can lead to excess formation of reactive oxygen species such as H_2O_2 and OH. The cytochrome P_{450} system plays a role in the detoxification of toxins in the body. However, in some cases, the product of the p_{450} enzymes are free radicals which may be more damaging than the primary toxin and cause oxidative stress. Excessive activation of phagocytic cells is an important cause of oxidative stress. Activated phagocytes produce different reactive species that may impose oxidative stress on tissues. This particular process has been implicated in many chronic diseases such as inflammatory based diseases and rheumatoid arthritis. Direct exposure to toxins from environment may also play a part in the generation oxidative stress. For example, cigarette smoke in the lung exposes lung tissues to free radicals. Tissue damage can be a cause of oxidative stress but oxidative stress can also be a cause of tissue damage (1-4). On the basis of leads available from folk usage and recent experimental and clinical studies *Glinus oppositifolus, Erythrina stricta* and *Trigonella foenum-graeceum* has been selected to screen for their possible *in vitro* and *ex vivo* antioxidant activity. *Glinus Oppositifolous* distributed in India to southeast Asia and the whole parts of this plant are used for immune response. The ethanolic fraction of this plant has been reported for antifungal, antiprotozoal activity. *Erythrina stricta* used as an antidote to poison, also used for rheumatism, leprosy, epilepsy, fever and itching sensation. *Trigonella foenum-graecum* possess antidiabetic, hypolipedemic, rheumatism and dyspepsia. The alcoholic extract of this plant has reported for antidiabetic, hypolipidemic activity. Based on their uses and the mechanism suggested for the prognosis of the diseases could be due to the inherent antioxidant property of the plant candidates of interest. In this present study it is proposed to evaluate the antioxidant activities of the selected plants against various *in vitro* antioxidant activity models.

MATERIALS AND METHODS

Collection and authentication

Glinus oppositifolius, Erythrina stricta and *Trigonella Foenum-Graecum* plants were collected from East godavari district, Andhra Pradesh.

Extraction of the powdered plant material

All the three (*Glinus oppositifolius, Erythrina stricta*, and *Trigonella foenum-graecum*) plants were dried under shade separately and powdered in mechanical grinder. The powdered plant materials were then passed through a sieve No 22 and stored in air tight container for further use. Extraction was carried out using ethanol by a simple maceration technique. Seven hundred and fifty milliliter (750 ml) of ethanol was added to 75 g of powder and kept on mechanical shaker for 4 h and filtered through Whatmann No.1 filter paper. The filtrate was evaporated under reduced temperature and pressure to constant weight. The percentage yield of ethanolic extract was calculated for each plant.

In Vitro Methods Employed In Antioxidant Studies

DPPH radical scavenging assay (5)

The hydrogen donating ability of extracts were examined in the presence of DPPH stable radical. Sample extract and standard stock solution (1mg/ml) was diluted to final concentrations of 1, 2, 4, 8, 16 µg/ml in ethanol. One millilitre of 0.3 mM DPPH ethanol solution was added to 2.5 ml of sample solution of different concentration and allowed to react at room temperature. After 30 minutes the

absorbance values were measured at 517 nm. The values obtained were converted into percentage antioxidant activity (AA%) using the following formula.

$$AA\% = 100 - \left[\frac{\left[\left(Abs_{sample} - Abs_{blank}\right)\times 100\right]}{Abs_{control}}\right]$$

Ethanol (1.0 ml) plus plant extract solution (2.5ml) was used as a blank, DPPH solution (1.0ml, 0.3 mM) plus ethanol (2.5ml) served as negative control. The positive controls were those using the standard (Ascorbic acid) solutions. The IC_{50} values were calculated by linear regression of plots, where the abscissa represented the concentration of tested plant and the ordinate the average percent of antioxidant activity from mean of three separate tests.

Nitric oxide radical scavenging assay(6)

The reaction mixture with a final volume of 3 ml per tube contained 2.0 ml of 10 mM sodium nitroprusside in standard phosphate buffer solution and was incubated with 1.0 ml of different concentrations of test compound dissolved in phosphate buffer (0.025 M, pH 7.4) and the tubes were incubated at 25^0C for 150 min. Control experiments without the test compounds but with equivalent amount of buffer were prepared in the same manner as done for the test. There after, 0.5 ml of incubation solution was removed and diluted with 0.5 ml Griess' reagent (1% sulphanilamide, 2% O-Phosphoric acid and 0.1% naphthyethylene diamine dihydrochloride) and allowed to react for 30 min. The absorbance of the chromophore formed during diazotisation of nitrite with sulphanilamide and subsequent coupling with naphthyethylene diamine dihydrochloride was read at 546nm. The percentage inhibition was calculated by using the formula

$$\text{Percentage inhibition (I)} = \left[\frac{\left(A_0 - A_1\right)}{A_0}\right]100$$

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance of the test compound. The experiment was done in triplicate using curcumin (50-800 µg/ml) as positive control.

RESULTS AND DISCUSSION

Glinus oppositifolius (whole plant), *Erythrina stricta* (leaves), and *Trigonellafoenum-graecum* (seed) were taken and extracted with water and 70% ethanol (30:70) and the yield of alcoholic extract was found to be 17% w/v, 7.2% w/v, and 4.1% w/v. This extract was used to carry out the *in vitro* and *ex vivo* antioxidant activity.

The ethanol extracts of *Glinus oppositifolius* (GOEE), *Erythrina stricta* (ESEE), *Trigonella foenum-graecum* (TFGEE) demonstrated H-donating activity. The radical scavenging activity of GOEE was determined from the reduction in absorbance at 517 nm due to scavenging of stable DPPH free radicals. The DPPH scavenging potential for GOEE varied at varying concentrations (100, 200, 400, 800, 1600 µg/ml). GOEE showed the graded increase in percentage of inhibition for all the doses tested and the percentage inhibition ranged from 3.4 to 78.4 %. The IC_{50} value of GOEE was found to be 1000 µg/ml. The free radical scavenging effect of ESEE was checked at doses ranging from 25 – 400 µg/ml and increase in percentage of inhibition could be noticed in all the doses used in the study. The dose of 25 µg/ml solution of ESEE gave a percentage inhibition of 25.63 and the same at 400 µg/ml exhibited 96.84 % and the IC_{50} value was 80 µg/ml. The percentage of inhibition for TFGEE at doses of 100, 200, 400, 800, 1600 µg/ml ranged from 16.23 for the lower doses and 71.05 for the higher dose with IC_{50} value of 800 µg/ml. Ascorbic acid was used as reference material and increase in percentage of inhibition was observed for all the concentration (1 – 16 µg/ml) tested. . Of the three plants tested, ESEE could offer greater percentage of inhibition at doses of 25 – 400 µg/ml when compared with GOEE and TFGEE used in doses of 100 -1600 µg/ml. The results were found to be statistically significant ($P<0.01$) (Table-1). The reductive ability of the extract served as a significant indicator of its potential antioxidant activity. Table-2 shows the reducing capacity of GOEE, ESEE, TFGEE compared with BHT. The reducing power of GOEE increased with increasing amount of the sample. GOEE increased the absorbance up to 0.785 at 800 µg/ml and the reference compound BHT showed the absorbance of 1.092 at 800 µg/ml. All three plant extract and standard were used a dose range of 50 – 800 µg/ml .All concentrations of the extract offered higher absorbance values than control and these differences were significant ($P<0.01$ Vs control).

Table-1 Hydrogen donating ability of plant extract

Sample	Concentration (μg/ml)	% inhibition	IC_{50} (μg/ml)
GOEE	100 200 400 800 1600	3.4±0.01 8.6±0.007 19.0±0.008 39.85±0.009 78.4±0.003	1000
ESEE	25 50 100 200 400	24.63±0.01 33.15±0.002 61.34±0.004 74.21±0.011 96.84±0.004	80
TFGEE	100 200 400 800 1600	16.23±0.003 19.88±0.004 31.05±0.004 50.11±0.002 71.05±0.001	800
Ascorbic acid (Standard)	1 2 4 8 16	0.36±0.01 25.73±0.015 73.41±0.007 91.11±0.002 94.05±0.004	3.1

Table-2 Reductive ability of plant extract

Sample	Concentration (μg/ml)	Absorbance
GOEE	50 100 200 400 800	0.436±0.002 0.452±0.005 0.495±0.008 0.568±0.005 0.785±0.032
ESEE	50 100 200 400 800	0.062±0.001 0.097±0.001 0.167±0.002 0.225±0.003 0.332±0.004
TFGEE	50 100 200 400 800	0.181±0.005 0.221±0.03 0.252±0.002 0.274±0.004 0.354±0.01
BHT (Standard)	50 100 200 400 800	0.092±0.002 0.214±0.004 0.314±0.004 0.640±0.02 1.092±0.005

CONCLUSION

Based on the mechanism of reduction of DPPH molecule described in the literature that is correlated with the presence of hydroxyl groups on the antioxidant molecule we can infer that the very good activity of ethanolic extract of *Glinus oppositifolius*, *Erythrina stricta* and *Trigonella foenum-graecum* is probably due to the presence of substance with an available hydroxyl groups. The reductive capabilities of the ethanolic extracts of *Glinus oppositifolius*, *Erythrina stricta* and *Trigonella foenum-graecum* were compared with butylated hydroxy toluene (BHT) for the reduction of the Fe^{3+} - Fe^{2+} transformation in the presence of the ethanolic extracts of the plants. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

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