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IN VITRO ANTI-OXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *GREWIA FLAVESCENS* WHOLE PLANT EXTRACT

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Abstract:

Objective: *Antioxidants or inhibitors of oxidation are compounds which retard or prevent the oxidation and in general prolong the life of the oxidizable matter. So, the objective of our work is to investigate the antioxidant activity using ethanolic extract of whole plant of Grewia flavescens.*

Methods: *The extract was screened for possible antioxidant activities by free radical scavenging activity (DPPH) and by lipid peroxidation method.*

Results: *The results showed that the plant possessed antioxidant properties including radical scavenging, and Percentage inhibition by lipid peroxidation. The extract of Grewia flavescens was effective against oxidant activity and the results were almost close to standard.*

Conclusions: *This study suggests that Grewia flavescens extracts exhibit great potential for antioxidant activity and may be useful for medicinal functions.*

Keywords: *Grewia flavescens, antioxidant, Plant extract.*

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INTRODUCTION:

The free radicals may be either Oxygen derived (ROS) or Nitrogen derived (RNS).[1] The most common reactive oxygen species include superoxide anion (O2),[2] hydrogen peroxide (H_2O_2), peroxyl radicals (ROO) and reactive hydroxyl radicals (OH). The nitrogen derived free radicals are nitric oxide (NO), peroxy nitrite anion (ONOO),

Nitrogen dioxide (NO_2) and Dinitrogen trioxide (N_2O_3). The exogenous sources of ROS include electromagnetic radiation, cosmic radiation, UV-light, ozone, cigarette smoke and low wavelength electromagnetic radiations and endogenous sources are mitochondrial electron transport chain, β-oxidation of fat. Chemical compounds and reaction capable of generating potential toxic oxygen species/free radicals are referred to as 'pro-oxidants'. They attack macromolecules including protein, DNA and lipid causing to cellular/tissue damage on the other hand, compounds and reactions disposing of these species, scavenging them suppressing their formation or opposing their actions are called antioxidants. In a normal cell there is an appropriate pro-oxidant: antioxidant balance. However, this balance can be shifted towards the pro-oxidant when production of oxygen species is increased or when levels of antioxidants are diminished. This state is called 'oxidative stress' and can result in serious cell damage if the stress is massive or prolonged.

Herbal antioxidants have been successfully employed as rejuvenators, for several centuries in the Indian systems of alternative medicine. Generally free radicals are produced in large amounts during metabolic diseases conditions like diabetes, hypertention, atherosclerosis, urolithiasis, ulcers etc., they may cause damage to the organs and ultimately leads to fatal effects if the production of the free radicals is not controlled.

Free radicals are a molecule with an impaired electron. Normally it steals an electron from a weakly bonded structure. The molecules, which losses an electron also, becomes a free radical giving rise to a self-perpetrating chain system. Free radicals often attack DNA, protein molecules, enzymes and cells leading to alterations in genetic material and cell proliferation (tumor masses). A majority of carcinogenic agents are regarded as powerful generators of free radicals, which initiate chain reaction and damage the cells and its components.

When the normal level of antioxidant defense mechanism is not sufficient for the eradication of free radicals induced injury, administration of antioxidants has a protective role to play. several antioxidants of plant origin are experimentally proved and used as effective protective agents against oxidative stress. They play an important role in major health problems such as cancer, cardiovascular diseases, rheumatoid arthritis, cataracts, Alzheimer's disease and degenerative diseases associated with aging [3-8]

There are various plants which are used to treat the diseases like ulcers, diabetes, epilepsy and cardiovascular diseases. One such plant is *Grewia flavescens* which was reported to cure diseases like diabetes, syphilis, ulcers, piles, lung diseases, and biliousness that are caused due to stress conditions. It contains various chemical constituents like steroids, alkaloids, flavanones, coumarins, reducing sugars, tannins, and proteins[9].
Hence an attempt has been made to evaluate the antioxidant property.

METHODOLOGY AND RESULT:

Plant collection and authentication: The crude *Grewia flavescens* juss. whole plant was collected from Sri Venkateshwara university, Tirupathi, Andhra Pradesh, India. The plant was authenticated with plant voucher specimen no. 1397, by plant taxonomist Dr. K. Madhava chetty, Assisstant professor, Department of botany, Sri Venkasteshwara university, Tirupathi, Andhra Pradesh, India.

Preparation of plant extract: The fresh whole plant of *Grewia flavescens*. were collected in the month of December from Tirupathi, India. The whole plant *Grewia flavescens* was washed with tap water, shade dried for two weeks, and pulverized. Then passed through sieve number 60 and stored in an air tight container. About 1000g of powered drug extracted with 80% ethanol by using maceration method of extraction. The method is followed until the phytoconstituents were completely exhausted. The ethanolic extract was evaporated through rotary evaporator under reduced pressure at 400C and labeled as EEGF 6.2% yield and preserved at 50 C in airtight container until further use.

Determination of free radical scavenging activity by DPPH method[10-12]:
Principle: This is one of the widely used methods for screening of anti-oxidant activity of plant drug DPPH assay method is based on reduction of absorbance of ethanol solution of DPPH by free radical scavenger.
Reagents required:

1. 1.1-diphenyl-2-picryl hydrazide (DPPH) (sigma chemicals UAS)

2. Dimethyl sulphoxide (DMSO) (sigma chemicals UAS)
3. Extract (20,40,60,80,100µg) in DMSO
4. Ascorbic acid (20,40,60,80,100µg)
5. Ethanol

Procedure: DPPH scavenging activity was measured by the spectrophotometric method. A stock solution of 25 mg of DPPH (150µm) was prepared in 100ml of ethanol. To the 0.2ml of EEGF extract of different concentrations, 3.8ml of DPPH was added. Control without test compound was prepared in an identical manner. In case of blank, DPPH was replaced by ethanol. The reaction was allowed to be completed in the dark for about 20 minutes. Then the absorbance of test mixtures was read at 517nm. The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical ascorbic acid (20,40,60,80,100 µg/ml). was used as standard. The percentage DPPH inhibition was calculated from the following formula.

% DPPH inhibition = O D of control - O D of test / O D of cont X 100

PERCENTAGE INHIBITION BY DPPH METHOD:

Table 01: Effect of different conc. of extract and standard on DPPH percentage inhibition

S.NO	CONC. (µG/ML)	% INHIBITION
1	Control	10.3 ± 0.89
Standard (Ascorbic acid)		
2	20	65.4 ± 0.70
3	40	72.5 ± 0.42
4	60	77.1 ± 0.57
6	80	82.03 ± 0.2
7	100	90.7±0.57
EEGF		
8	20	24.25 ± 1.97**
9	40	29.01 ± 1.45**
10	60	44.07 ± 0.48**
11	80	59.24 ± 1.34**
12	100	72.1 ± 0.47* *

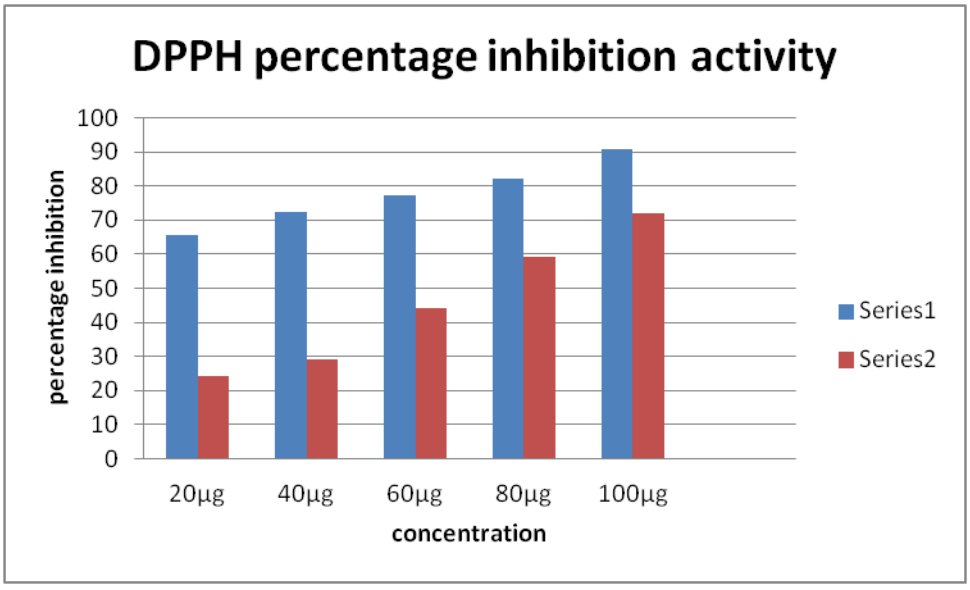

Figure 01: Percentage inhibitions by DPPH method.

In the present study EEGF at different concentration were assessed for their free radical scavenging activity in an in vitro model. It was observed that % inhibition of EEGF scavenged free radical in a dose dependent manner. All the concentration showed significant increase in the DPPH percent inhibition. So the plant extract under investigation are potent inhibitor of DPPH.

Determination of lipid peroxidation activity[13-14]:

Principle: The lipid in the cell membranes are highly susceptible to peroxidative damage and are broken down into number of small unit to form malandialdehyde. This reacts with thio barbituric acid (TBA) to form thiobarbituric acid reacting substances (TBARS) which has a pink colour with absorption maxima at 532nm.

Reagents required:

1. Potassium chloride (0.15 , PH 7.4)
2. Liver homogenate 10%(oil ml)
3. Ferrous sulphate (25 m , 0.1ml)
4. KH_2PO_4(10mM,0.1ml)
5. Extract (20,40,60,80,100g) in DMSO
6. Ascorbic acid (20,40,60,100mg)
7. Trichloro acetic acid (15%, 1ml)
8. Thiobarbituric acid (0.375% ,0.5ml)

Procedure: Liver homogenate was prepared from the Wister albino rats. Liver was quickly excised after decapitation and exsanguinations and washed several times with ice cold saline solution (0.15M KCl, PH 7.4), then homogenized in the same saline solution. A 10% liver homogenate was prepared and the test system contained homogenate with a protein concentration of 500 µg / ml lipid peroxidation was initiated by the ascorbic acid 10Mm KH_2PO_4 and liver homogenate. The homogenate, were incubated at 37ºC for one hour with different concentrations of extract. To the test tube 1ml of TCA and 0.5ml of 0.375% TBA were added. The tubes were placed in boiling water bath for 30 min. Then centrifuged and then the supernatant was measured at 532 nm. The percentage lipid peroxidation incubation was calculated from the following formula.

% Lipid peroxidation inhibition = OD of control – OD of test / OD of control X 100.

LIPID PEROXIDATION ACTIVITY:

Table no 02: Effect of different concentration of EEGF whole plant extract and standard on Lipid peroxidation activity.

S. no	Conc. (µg/ml)	% Inhibition
1	Control	6.30 ± 0.59
Standard (Ascorbic acid)		
2	20	33.70 ± 0.60
3	40	70.55 ± 0.82
4	60	73.48 ± 0.67
5	80	84.11 ± 0.25
6	100	95.77 ± 0.37
EEGF		
7	20	20.34 ± 0.95**
8	40	24.95 ± 0.45**
9	60	56.70 ± 0.48**
10	80	77.18 ± 0.34**
11	100	92.50 ± 0.57* *

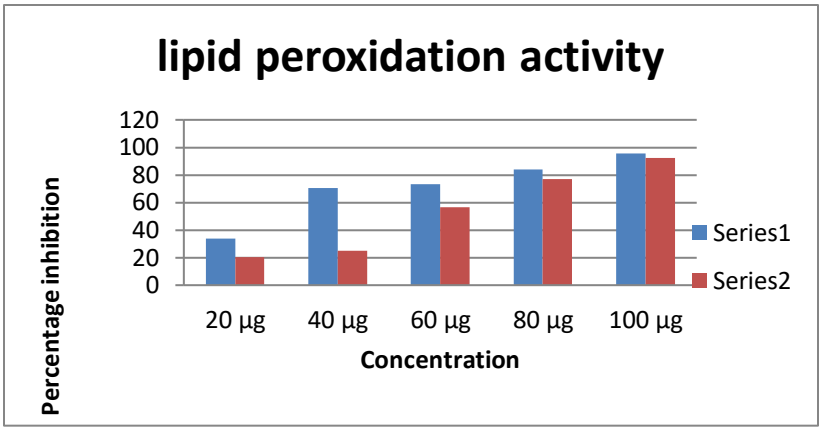

Figure 02: Percentage inhibition by lipid peroxidation

The different concentration of the sample was compared with the standard of similar concentration significant difference was found in the activity. The 100-µg concentration of the extract showed significantly high antioxidant activity than other concentration when compared to standard. All the concentration of extract showed significant difference in the lipid per oxidation inhibition when compared to standard.

DISCUSSION:

In the present study EEGF at different concentration were assessed for their free radical scavenging activity in an in vitro model. It was observed that % inhibition of EEGF scavenged free radical in a dose dependent manner. All the concentration showed significant increase in the DPPH percent inhibition. So the plant extract under investigation are potent inhibitor of DPPH. The different concentration of the sample was compared with the standard of similar concentration significant difference was found in the activity. The 100 µg concentration of the extract showed significantly high antioxidant activity than other concentration when compared to standard. All the concentration of extract showed significant difference in the lipid per oxidation inhibition when compared to standard.

CONCLUSION:

In the present study, analysis of radical scavenging abilities and inhibited lipid oxidation showed that ethanolic extract of *Grewia flavescens* could be a potent source of natural antioxidants. To the best of our knowledge, this is the first study to suggest that ethanolic extract of *Grewia flavescens* may be useful as a natural antioxidant due to its antioxidant capacities.

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