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A STUDY ON PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF GREWIA TENAX

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Abstract: Phytochemical analysis of Grewia Tenax plant samples revealed presence of Alkaloids, Amino acids, Carbohydrates, Glycosides, Anthraquinone, Saponin, Tannin and Triterpenoids. n-Hexane, Ethanon and Hydro alcohol solvent extracts of Grewia Tenax were tested for their antioxidant and cytotoxic activity. Positive antioxidant activity but with seasonal variation was found for all the extracts using TLC based standard qualitative 2,2 diphenyl-1-picrylhydrazys(DPPH) assay and hydrogen peroxide radical scavenging activity. However, a seasonal variation was observed in all the extracts. February and October were found to be favorable for the presence of antioxidant property. n-hexane extract of Grewia Tenax showed maximum antioxidant bands (25) and promising antioxidant activity at par with the standard antioxidant ascorbic acid (IC50 9.5microgram). Brine shrimp motility assay at the doses of 25 and 50microgram/ml showed variation in plant extracts activity. The potency of methanol extract of the plant showed rich antioxidant properties, so same was fractionated and a pure antioxidant fraction was isolated which showed good antioxidant activity.

Keywords: Antioxidant, Anthraquinone, Saponin, alkaloids, flavonoids, tannins, saponins, grewia tenax, DPPH, hydrogen peroxide radical scavenging activity

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INTRODUCTION

Anti-oxidants have been found to be of assistance for the cure of lipid metabolism, atherosclerosis, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative and cardiovascular disease in hemodialysis patients (Young and Woodside, 2001)[1]. Antioxidants act as scavengers preventing cellular and membrane damage. This progression is called anti-oxidation. The major natural antioxidants include vitamin E, vitamin C (ascorbic acid), polyphenols, bioflavonoids and carotenoids [2]. Antioxidants act in apathetic ways by preventing free radical formation (metal chelation), by scavenging free radicals preventing the proliferation of the oxidative chain reaction, by being part of the redox antioxidant network, or by regulating gene expression. Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in re-antioxidants in reducing oxidative stress-induced tissue injury (Pourmorad *et al.,* 2006). Among the abundant naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective (Duh *et al*., 1999). They are known to inhibit lipid peroxidation (by inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions (Sundar rajan *et al.,* 2006). The study done on medicinal plants strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress (Cao *et al*., 1996). The free radical scavenging activity against 1,1-diphenyl-2-picryl hydrazyl (DPPH) was evaluated during the course of work. The ascorbic acid, carotenoids and total phenol contents with antioxidant activity were also determined.

II. MATERIALS AND METHODS

2.1. COLLECTION OF PLANT MATERIAL

Ethnobotanical survey on medicinal plants found in the Ananthapuramu district was extensively studied and two folklore medicinal plants Grewia Tenax were identified for the present investigation [3]. Based upon the information collected on the medicinal plant were collected, identified and authentification was made from the local botanist.

The specimens were poisoned by dipping the whole twig in the Ethyl alcohol saturated with Mercuric Chloride solution and immediately placed between the blotting papers with forceps. These were tied properly in iron press with the help of a rope. After 12 hours, the blotting papers were changed and specimens were spread properly [4]. Some leaves were placed facing upwards and some facing downwards to show the characters on both surfaces. These spread specimens were once again placed in other dry blotting sheets and tied again and this process was continued until the specimen dried completely (Jain and Rao 1977), Poisoned, pressed and dried specimens were pasted with glue on thick amount boards measuring 42X28 cm and stitched. Labels containing all the information viz., name of the plant, family, locality, altitude, date of collection, notes as in field book and collectors name were affixed on the right hand bottom corner of the mounted sheets[5]. The mounted specimens were preserved in steel almaras following Bentham and Hooker's system of classification.

III. PHYTOCHEMICAL ANALYSIS

Phytochemical analysis was conducted using standard protocols Sofowora, 1993 and Trease and Evans., 1997). A brief account of the different tests conducted was as follows:

Alkaloids: 1 ml of methanolic extract of Grewia Tenax was filtered. Then 2 ml of 1% aqueous HCl was added to it. Thereafter it was heated for few minutes [6]. 2 drops of dragondroff

reagent was added to the solution. Reddish brown precipitate with turbidity depicts alkaloid's presence.

3.1. FLAVONOIDS

To 5 ml of methanolic extract of Grewia Tenax, 1 ml of 10% NaOH solution was added. From the side of the beaker 2 drops of concentrated HCl was added. Yellow colour turning to colourless is an indication of presence of flavonoids.

3.2. SAPONINS

About 2 ml of 1% sodium bicarbonate was added to 1 ml of methanolic bark extract of Grewia Tenax and shaked. Lather like formation persistent for some time is indicative of presence of Saponins.

3.3. GLYCOSIDES

100 µl methanolic extract of Grewia Tenax was taken in a test tube and 400µl of acetic anhydride was added to it. Then 1-2 drops of concentrated sulphuric acid was added to it. Blue-Green colour shows the presence of glycosides.

3.4. ANTHRAQUINONE

To 1 ml of methanolic extract of Grewia Tenax, 2 ml of 5% KOH was added. Then the solution was filtered. Change in colour was observed. Pink colour shows the presence of anthraquinones.

3.5. TANNIN

1gm of sample added with 100ml of distilled water, boiled and cooled, and then filtered. 1% ferric chloride was added drop wise to the filtrate. Green black precipitate shows the presence of tannin.

IV. PROCESSING OF PLANT MATERIAL FOR SOLVENT EXTRACTION

4.1. EXTRACTION PROCESS FOR THE SELECTED PLANT MATERIALS

The selected plant material Grewia Tenax was subjected for the hot non-sequential extraction process by using soxhlet apparatus [7]. The plant material was extracted using the different solvents (n-hexane, ethanol and hydro-alcohol system 1:1) by increasing in polarity.

4.2. PREPARATION OF EXTRACTS

The plant powders were subjected to serial solvent extraction and extracts n-hexane, methanol, hydroalcohol was prepared respectively. These extracts were further concentrated by using Buchi (R-200) Rotavapour. The concentrated semi-solid extracts were stored in air tight screw cap vials and kept in refrigerator till further use (Mohapatra *et al.*, 2010).

4.3. DPPH Free Radical Scavenging Activity

The plant extract was dissolved in small quantity of water to get a concentration of (1mg/mg), 2.5ml this solution was added with 1ml (0.1mM) DPPH (in ethanol) solution to get a serial dilution in the concentration ranging from (25 to 300µg/ml) with ethanol[8] as shown in Table.1. The resulting solution as shown in fig.1 was incubated in the room temperature for 20 minute and the all the solution was measured at 517nm spectrophotometricaly against the reagent blank. Ascorbic acid was used as the reference compound [9]. The IC50 and percentage inhibition was calculated by using the following equation.

$$\% I \text{ (percentage inhibition)} = [(Ac - At)/ At] \times 100$$

Where, Ac = was the absorbance of the negative control

(Blank, without extract or standard),

At -was the absorbance of all the extract and reference compound.

Table.1: DPPH radical scavenging activity of Grewia Tenax

Extract / Standard	Conc. (µg/ml)	Absorbance (MEAN ± SD) n=3	% Inhibition (MEAN ± SD) n=3	IC_{50} (µg/ml)
Control	- -	0.627	- -	--
Ascorbic Acid	25	0.978 ± 0.002	45.056 ± 0.112	19.933
	50	0.877 ± 0.003	50.730 ± 0.195	
	100	0.741 ± 0.005	58.371 ± 0.297	
	150	0.676 ± 0.004	62.004 ± 0.213	
	200	0.553 ± 0.002	68.951 ± 0.086	
	300	0.456 ± 0.011	74.382 ± 0.618	
n-Hexane extract of Grewia Tenax	25	0.620 ± 0.001	1.116 ± 0.159	188.561
	50	0.594 ± 0.004	5.210 ± 0.645	
	100	0.555 ± 0.005	11.483 ± 0.797	
	150	0.520 ± 0.001	17.065 ± 0.159	
	200	0.493 ± 0.003	21.425 ± 0.487	
	300	0.471 ± 0.001	24.934 ± 0.184	
Etyhanolic extract of Grewia Tenax	25	0.969 ± 0.040	45.562 ± 2.241	26.507
	50	0.897 ± 0.004	49.607 ± 0.203	
	100	0.830 ± 0.005	53.352 ± 0.265	
	150	0.772 ± 0.005	56.629 ± 0.297	
	200	0.640 ± 0.006	64.064 ± 0.309	
	300	0.531 ± 0.005	70.169 ± 0.297	
Hydro alcoholic extract of Grewia Tenax	25	0.584 ± 0.005	6.858 ± 0.829	79.250
	50	0.501 ±0.003	20.043 ± 0.401	
	100	0.458 ± 0.001	26.954 ± 0.159	
	150	0.395 ± 0.005	37.055 ± 0.787	
	200	0.322 ± 0.002	48.618 ± 0.322	
	300	0.226 ± 0.004	64.009 ± 0.604	

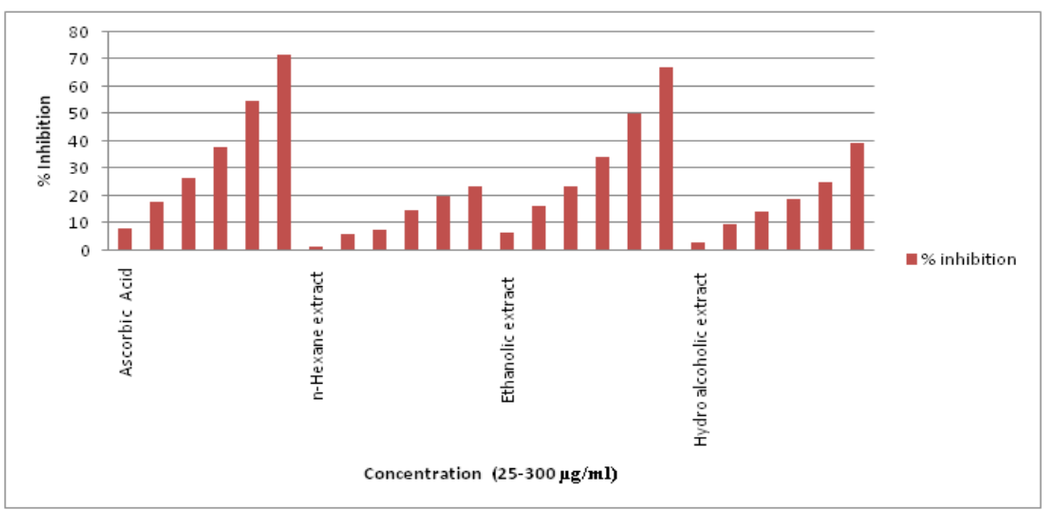

Fig.1: DPPH radical scavenging activity of Grewia Tenax

V. HYDROGEN PEROXIDE ANTIOXIDANT ACTIVITY

40mM of hydrogen peroxide solution was prepared using phosphate buffer solution (pH 7.4). Extract was dissolved in small quantity of distilled water to get a concentration of (100 µg/ml). This solution was added to 0.5ml of 40mM solution of hydrogen peroxide to get a concentration ranging from (25 - 300µg/ml) was made adjusted using distilled water as shown in Table.2. This resulting solution as shown in fig.2 was measured at 230nm and the percentage inhibition was determined by using the above mention formula, Gallic acid was used as reference standard.

Table 2: Hydrogen peroxide scavenging activity of Grewia Tenax

Extract / Standard	Conc. (µg/ml)	Absorbance (MEAN ± SD) n=3	% Inhibition (MEAN ± SD) n=3	IC_{50} (µg/ml)
Control	- -	0.627	- -	--
Gallic Acid	25	0.342 ± 0.006	7.975 ± 1.728	197.087
	50	0.305 ± 0.004	18.100 ±1.086	
	100	0.272 ± 0.004	26.792 ± 1.018	
	150	0.231 ± 0.006	37.814 ±1.728	
	200	0.168 ± 0.001	54.839 ±0.269	
	300	0.105 ± 0.007	71.774 ±1.882	
n-Hexane extract of Grewia Tenax	25	0.363 ± 0.004	2.509 ± 1.086	437.634
	50	0.336 ± 0.005	9.767 ± 1.379	
	100	0.310 ± 0.003	12.756 ± 0.677	
	150	0.294 ± 0.006	20.968 ±1.635	
	200	0.278 ± 0.003	26.047 ± 0.677	
	300	0.237 ± 0.005	29.201 ± 1.326	
Etyhanolic extract of Grewia Tenax	25	0.350 ± 0.006	5.914 ± 1.497	167.424
	50	0.307 ± 0.004	17.473 ± 1.172	
	100	0.273 ± 0.005	26.523 ± 1.353	
	150	0.238 ± 0.004	36.111 ± 1.086	
	200	0.175 ± 0.005	52.957 ± 1.232	
	300	0.096 ± 0.006	74.104 ± 1.529	
Hydro alcoholic extract of Grewia Tenax	25	0.352 ± 0.005	5.287 ± 1.270	242.021
	50	0.322 ± 0.002	13.441 ± 0.538	
	100	0.301 ± 0.003	19.176 ± 0.677	
	150	0.277 ± 0.003	25.538 ± 0.711	
	200	0.241 ± 0.004	35.305 ± 1.018	
	300	0.180 ± .005	51.613 ± 1.232	

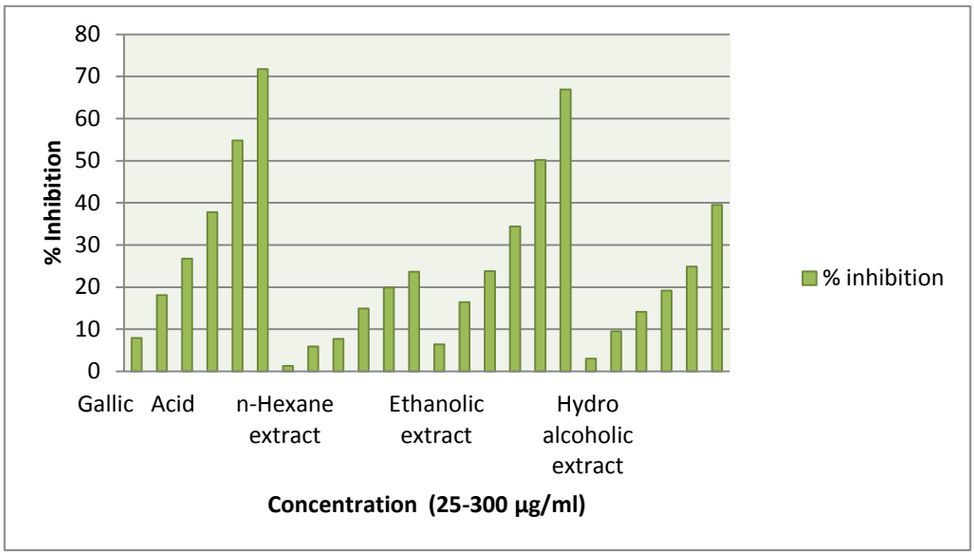

Fig.2: Hydrogen peroxide scavenging activity of Grewia Tenax

VI. RESULTS AND DISCUSSIONS

Phytochemical tests of the plant Grewia Tenax showed the presence of alkaloids, amino acids, carbohydrates, anthraquinon, saponin, tannin, flavonoids and triterpenoids. Out of these flavonoids is a class of compounds which are known for their medicinal potential [10]. In general they have been reported for their antioxidant, (Gretchen *et al.,* 2008: McGaw *et al.,* 2001). Detection of flavonoids in the plant species was expected as in another species rivastava *et al.,* 1999).

Antioxidant activity for the various crude extract of Grewia Tenax were subjected for DPPH and hydrogen peroxide model for the identification of the antioxidant activity of the crude extracts. The reactive oxygen species (ROS) is important biochemical process which involves in activation of the diseased condition such as cancer, alzheimer's, cardiac problems and Parkinson. The living bio system, cell prevents this ROS formation by producing biochemical changes to form antioxidants which in turn inactivate the reactive oxygen species, but the formation of antioxidant is not an simple task for the biological cell since the production of antioxidant is diminished by many factor Many of the antioxidants are obtained for the natural products from plants and marine sources [11]. Crude extract showed a potent antioxidant activity for the ethanolic extracts with the IC50- 26.507other extract possessed a moderate activity. For crude extract of grewia tenax the ethanolic extract showed a potent DPPH radical scavenging activity the IC50-56.477 and other extract showed a moderated antioxidant. All the extracts were compared with the standard ascorbic acid which produces an IC50 value of 19.933.

The hydrogen peroxide radical scavenging activity was determined for the crude extract of Grewia Tenax among all the extract the ethanolic extract of both the crude extract showed an potent activity on hydroxyl. The ethanolic extract of Grewia Tenax was found to be167.424 and 245.206. The hydro- alcoholic extract of both the plants showed a moderate and n-hexane extract showed and less activity when compared with the other extract and the standard compound gallic acid (IC50- 197.087).

From the antioxidant activity of the crude extract of Grewia Tenax were clearly show that the ethanolic extract of both the plants showed an potent antioxidant activity for the both the models (DPPH and hydrogen peroxide) since these crude extract were consist of some

common phytoconstitution such as saponin, tannin, and triterpenoids. These phytoconstitution have shown a strong antioxidant activity. Hence form the antioxidant activity was the ethanolic activity was found to be potent when compared with the respective reference standard ascorbic acid and gallic acid.

VII. DISCUSSION

Interest in the use of medicinal plants as insecticides has increased over a decade due to environmental concerns and development of resistance to the synthetic insecticides in insects. Naturally occurring insecticides are derived from plants source. The insecticidal constituents of various medicinal plant extracts and essential oils present in them are mono-terpenoids. Due to their high volatile nature they have significant insecticidal activity that might be of utility for controlling stored-product insects Drug.

Free radicals play a significant role in a wide variety of pathological conditions. Antioxidants can fight against free radicals and protect us against numerous diseases. They produce their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. Medicinal plants having vitamin C, E, carotenoids, flavonoids, polyphenols possess remarkable antioxidant activity that is responsible for inhibiting or preventing the harmful effects of oxidative stress. The imbalance between antioxidant defense systems and free radicals may cause damage to Anti-oxidant activity of the eight extracts was measured by DPPH free radical scavenging method and their scavenging activity was compared with the standard antioxidant ascorbic acid. The DPPH method is a simple, rapid and convenient method for screening of many samples for radical scavenging activity. The electron donation ability of natural products can be measured by 2, 2'-diphenyl-1-picrylhydrazyl radical (DPPH) purple-coloured solution bleaching. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test.

The phosphomolybdate method is a quantitative method of analysis, since the total antioxidant capacity (TAC) is expressed as ascorbic acid equivalents. The antioxidant capacity of the fractions was measured using spectrophotometer by phosphomolybdenum method, based on the reduction of Mo (VI) to Mo (V) by the test sample and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 765 nm. Researches revealed that many flavonoid and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants.

The results of total anti-oxidant activity revealed following results Potent anti-oxidant activity of Grewia Tenax extract may be attributed to its flavonoids, phenolic constituents, hydroquinone, its derivatives also possess significant antioxidant activity due to the presence of flavonoids, phenolic compounds. The pronounced antioxidant activity of Grewia Tenax may be due to the presence the flavonoid chrysoeriol in it . Graph 2 represents IC50 value of all extracts. The results of anti-oxidant activity reveals that medicinal plants can be effectively used due to their environmental friendly effects for the control of pest, storage of food and therapeutic effect against various pathologies at a lower cost and with lesser hazardous effects in comparison to the currently available synthetic drugs.

VIII. CONCLUSION

It can be concluded that the extracting solvent affects significantly the flavonoid compound content and the antioxidant activity measured and therefore it is recommended to use more than one extraction system for better assessment of the antioxidant activity of natural products. Several of the medicinal plants tested are rich sources of flavonoid compounds and free radical scavengers. Some medicinal plants thus can be considered as promising sources of natural antioxidants for medicinal and commercial uses.

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