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ANTIOXIDANT ACTIVITY OF CHLOROFORM EXTRACT OF *RUELLIA PROSTRATA*

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ABSTRACT

Leaves of *Ruellia prostrata* were subjected to extraction and further fractionation to obtain antioxidant rich fraction. The crude protein was isolated from Chloroform and Aqueous extract of Ruellia prostrata by SDS-PAGE. Different concentrations of chloroform and aqueous extract of Ruellia prostrata were used to assay the antioxidant activity by DPPH method, ilealoop assay. However, the chloroform fraction showed a good reducing power and better free radical scavenging activity. The phytochemical screenings confirm the presence of glycosides, lipids, alkaloids, and carbohydrates which are responsible for the antioxidant activity.

KEYWORDS: Antioxidant, Ruellia prostrata, Free radical.

INTRODUCTION

Their antioxidants effectiveness in food depends on not only the number and location of hydroxyl groups but also on factor such as physical location interaction with other food components and environment condition. In many studies phenolic compounds demonstrated higher antioxidant activity than antioxidant vitamins and carotenoids (Re et al.1999 and Veliglu *et al.*, 1998) The antioxidant properties of phenolic compounds are mainly because of their redox potential which allow them to act as reducing agents hydrogen donators, metal chelators and singlet oxygen quenchers (Rice-Evan *et al.*, 1996) It is known that the degree of glycosylation significantly affects the antioxidants properties of the compounds. (Marc hand. 2002). Flavanoids are naturally occurring phenolic compounds which largely include anthoxanthins (flavones, flavonols, flavanones, flavanols, chalcones and is flavones). Anthocyanins, leucozanthine and flavonoidal alkaloids (Houghton. 2002).

A variety of antioxidant defense system are including enzymatic and non enzymatic involved in the antioxidants. The main characteristics of an antioxidant is to trap free radicals. Highly reactive free radicals and oxygen speices are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acid, protein, lipids or DNA and can initiate degenerative diseases. Compounds like that lead to degenerative diseases.

Antioxidants are the one which dispose the reactive oxygen species, scavenge them suppress their formation, appose their action. They include the compounds like NADPH, GSH, ascorbir acid and vitamin c. when LDL is exposed to oxidation conditions the antioxidants present in the LDL react with the peroxides and quench the propogation of lipid peroxidation. The precise requirement of antioxidant to prevent the oxidation of LDL depends upon the nature of the LDL particle., fatty acid composition, The amount of the peroxides associated with LDL, the size of the particle, its lipid composition and other factors determine the rate of LDL oxidation (Buccolo G,etal.,1973)

The flavanoids contains a number of phenolic hydroxyl groups attached to ring structures which confer the antioxidant activity. (Chen and Chan., 1996).
DPPH radical scavenging activity and quercetin derivatives from this plant showed strong antiradical

activity (Braca *et al.*, 2002). The antioxidants activity of phenolic acids and their esters depends on the number of hydroxyl groups in the molecule and the activity can be strengthened by steric hindrances hydroxylated cinnamates are more effective than benzoate counter parts (Ricw-Evans *et al.*, 1996).

The antioxidant activities and total phenolic content of 30 samples of acacia honey from creation territory were analyzed phenolics were determined by the modified from (DPPH) method and potential antioxidant activity using the ferric reducing antioxidant power (FRAP) method. (M.krpan *et al.*, 2009). Herbal remedies may have recognizable therapeutics effects (Bailey *et al.*, 1989).The potent antioxidants that poses Antioxdative and antitumor activity (Ho *et al.*, 1994)

They also project plants from attack by microbes and insects. Flavanoids have been referred to as natures biological response modifiers because of their in herent ability to modify the bodys reaction to allergens viruses and carcinogen. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity(Miranda *et al.*, 2007).

Phytochemical review show the presence of flavonoid, glyucosides like apigenin 7-glucoside, luteoline, 7-glucoside, apigenin, 7β-glucuronide and mixture of sterols like stigmasterol, sitosterols, 24-methylcholesterol, cholesterol, brassicasterol, amino acids, and luperol mucilage also present. So it was found that it is of interest to study this plant and check out scientifically its uses in and folk medicine and traced any new biological activities.

MATERIALS AND METHODS

Collection of Plant

Ruellia Prostrata-Leaves were collected and taken to the laboratory, subsequently they were preserved at low temperature and portions of the specimens were preserved in 10% neutralized formalin for further identification. The samples were cleaned by using tap water and surface sterilization was done by NaOH.

EXTRACTION OF PLANT

Aqueous extraction

The aqueous extract of the sample was prepared by squeezing the sand – free specimens in triple distilled water. The resultant solution was filtered and dialyzed by using Sigma dialysis membrane-500 (Av Flat width-24.26 mm, Av. Diameter -14.3 mm and capacity approx-1.61ml/cm) against D-glucose to remove the excess water. The supernatant so obtained was lyophilized (Labcono Freeze Dry System) and stored at 4 ^{o}C in a refrigerator for the further use as crude aqueous extract.

Chloroform extraction

Crude was extracted following the method with certain modifications, for Chloroform extraction, the sample was dried in air for 2 days and after complete drying, 10 g sample was put into 200 ml of chloroform, covered and kept standing for 5 hours. The solvent was removed after squeezing the sample and filtered through Whatman filter paper No 1. The solvent was evaporated at low pressure by using a Buchi Rotavapor R-200 at 4^{o} C in refrigerator for further use as crude chloroform extract.

PREPARATION OF DEAE CELLULOSE COLUMN

26 gm of DEAE Cellulose was taken. It was swelled in 650ml of Distilled water and incubated for 3 hrs. Then the supernatant was discarded. The sediment was mixed with half a liter of 1M NaOH. This mixture was incubated for 30 minutes and again the supernatant was discarded. The collected sediment was mixed with half a liter of 1M HCL and it was incubated for 30 minutes and the supernatant was discarded. Then this was washed with distilled water and again with PBS till it reached pH 7.4.

PHYTOCHEMICAL ANALYSIS

Chloroform and aqueous fractions were subjected to phytochemical analysis for the presence of Alkaloids, Phenols, Tannins, Saponins, Carbohydrates, Lipids and Proteins.

PROTEIN ESTIMATION

Protein estimation was done as described by using Bovine serum Albumin at the rate of 1mg/ml as the standard. Different concentrations of the standard ranging from 0.1 to 1mg/ml were taken and made up to 1 mg/ml. Then 5ml of alkaline copper reagent was added, mixed well and allowed to stand for 10 minutes at room temperature. Then 0.5ml of diluted Folin's phenol reagent was added and mixed well. The mixture was incubated for 30 minutes at room temperature.The absorbance at 650nm was read spectrophotometrically. The protein concentrations of *Ruellia Prostrata* extracts were estimated.

ILEAL LOOP ASSAY

The effect of drug at lower and higher dozes in the intestine of the Mice was found by injecting 25µl/ml and 100 µl/ml to its intestinal wall. This was then stored in phosphate buffer saline (PBS). The extracts would attach to the acetyl choline receptor and the sodium potassium pump will be blocked as a result it brings out the fluid secretion. This proves that presence of toxin and the intensity of low dose can be used as therapeutic protein.

Antioxidant Assay: (DPPH assay)

Concentrate extracts was prepared with 0.1 mg/ml of crude extracts with and 50 µl of each extracts was loaded into 'U' bottomed 96 well plates to which 50µl of 0.1% DPPH (1,1 di-phenyl 2-picryl hydrazine) was added and then ascorbic acid (0.1 mg/ml) was used as the positive control and sterile water was used as negative control. The plate was incubated at dark for 30 minutes for the colour change. Development of Purple colour to yellow colour within 30 minutes indicated positive result.

CHARACTERIZATION OF PROTEIN

High Performance Liquid Chromatography (Hplc)

In HPLC, narrow columns with internal diameters 2-80 mm are used. These columns are packed with particles having an average diameter of less than 50 microns (50 x 10^{-6}m). Such columns require high pressures (1000-6000 psi) to maintain a convenient flow of the eluting solvent, usually in the range 0.1-10 mL/min, but occasionally higher. Resolution is considerably superior to that achieved with an ordinary column, in part because of the tight packing of the stationary phase, which reduces lateral diffusion, and because of the large surface area of the packing.

Compared with classical column chromatography, where the columns are gravity fed and a separation can take hours or even days, HPLC can offer analysis time of 5-30 min. Such times are comparable to that needed for GLC analyses.

HPLC is especially suited for the analysis of compounds not readily assayed by GLC. For example, thermally labile compounds can be analysed by HPLC at ambient temperatures, and highly polar or nonvolatile compounds can be analyzed. Sample treatment is often minimal since aqueous solutions can be used in HPLC.

THIN LAYER CHROMATOGRAPHY (TLC)

Thin Layer Chromatography (TLC) analysis was done. Commercially available TLC aluminum sheet (silica gel-60) was used as the supporting solid phase and Chloroform: ethanol: acetic acid: water (98:10:2:2) was used as the mobile phase. The developing agent used was Ammonium hydroxide with Silver Nitrate. The plate was kept at 105°C for 10 minutes in hot air oven. Development of blue and gray color was observed.

RESULT

PREPARATION OF CRUDE EXTRACTS

Chloroform extract of sample *Ruellia Prostrata* yielded a total amount of 11.5 of crude extract from 50g of sample.

PROTEIN ESTIMATION

The protein content in crude extract of *Ruellia Prostrata* was found to be 1.8mg/ml in case of chloroform extract. The results are presented in Table and as graphical representation below.

Antioxidant Assay: (DPPH ASSAY)

The DPPH reaction on both the extracts changed the colour which indicated the presence of antioxidant molecule supporting the thin layer chromatography result. DPPH is a useful reagent for investigating the free radical-scavenging activities of compounds. In the DPPH test, the extracts were able to reduce the stable radical DPPH to the yellow coloured 1-1diphenyl 2picrylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH by the reaction.

High Performance Liquid Chromatography (Hplc)

In the first set with a retention time of 4.37min, the percentage intension was found to be 25% for chloroform extract.

In the second set with a retention time of 6.75min, the percentage intension was found to be aqueous extract.

THIN LAYER CHROMATOGRAPHY

Four grey colored bands were observed in chloroform extract and one blue and one grey colored band was observed in act proving that antioxidant rich compounds were present in the sample.

Anti-oxidant is breaking the free radical chain reaction as a result of their ability to transfer hydrogen to a peroxyl free radical. This test was carried out to check if the samples contain anti-oxidant property so that the free radicals in the body can be converted to non free radical which is not toxic to the body. So the TLC was performed and the spray agent was a free radical so if our compound has anti oxidant property they would convert the free radical and give a colour like grey, black, brown indicating the breakage of the free radical. This was authenticated in **Harborne, (1967).**

TABLE – 1 Preparation of crude extract

S.No	Name of the solvent	Amount of crude extract yield (gm)
1	Chloroform	11.5
2	Aqueous	10.2

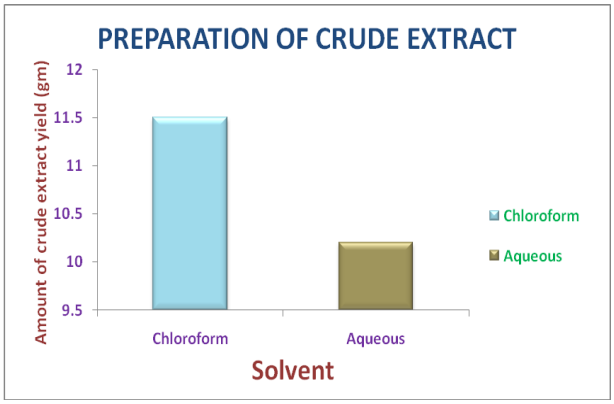

FIG-1

Table: 3.PROTEIN ESTIMATION

S.No	Type of extract	Concentration of protein(mg/ml)
1	Chloroform	1.8
2	Aqueous	2.9

PROTEIN ESTIMATION

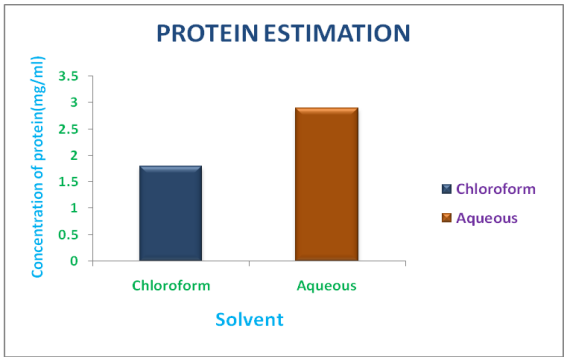

FIG-2

DISCUSSION

The sample identification was confirmed based on the morphological characters. Chloroform of sample *Ruellia Prostrata* yielded a total amount of 11.5g and10.8g of crude extracts respectively from 50 g of sample. The dry weight and wet weight ratio varied according to the biogeographical factors and its extraction yield differ from species to species of different herbal groups.

The protein contents of both chloroform extracts of *Ruellia Prostrata* was found to be 1.9mg/ml. Described that the higher protein was found in G. acerosa (31.07 ± 0.33%) followed by H. macroloba (28.94 ± 0.68%), H .tuna (23.12 ± 0.86) and C. glomerata (20.38 ± 0.73%).

The present results confirm to those of earlier studies; Stempein (1970) reported the hemolytic activity halitoxin of genus Haliclona. Fusetaniet *et al.*, (1981) Reported that the sterol derivatives from Halichondriidae sponges viz., Halistanol sulphate and sokotrasterol sulphate, Mebs *et al.*, (1985) reported the Haemagglutinaion and hemolytic activity of aqueous extract from 48 tropical sponge species. The increase in scavenging activity of *Ruellia Prostrata* extracts on DPPH radicals was dependent on concentration. The chloroform extract exhibited a strong scavenging activity on DPPH (95 and 82 %) at 0.25 mg concentration.

Lahaye and Kaffer (1997) explained that samples are low in fats but contain vitamins and bioactive compounds, like terpenoids, sulfated polysaccharides and polyphenolic compounds, the latter being a potential natural antioxidant not found in land plants. Le Tutour et al. (1990) investigated the antioxidant activities of different samples.

The chloroform had shown changes at the tissue level the was found to be non-lethal and had no pathological effects. From the present study it is quite evident that the chloroform extracted toxin from *Ruellia Prostrata* was highly in nature, which can affect the vital organs of vertebrate animals as evidenced in the present study where the Mice was used as the laboratory model. The SDS-PAGE on gel, crude protein yielded 3 bands in the chloroform extract t of *Ruellia Prostrata* ranging from 120 to 158 kDa molecular weight with 3 well-defined bands of 123, 132.3and 142.9kDa in both the extracts.

Yoshihiro et al. (1987) showed that SDS- PAGE patterns consisting of many protein bands were obtained for the three samples. In each sample, most fractions gave rise to patterns fairly similar to each other.

For first set of experiments performed with retention time of 4.37min, the percentage of intension decreased for chloroform extract. Thenmozhi *et al.*, (2008) observed that for retention time 4.37min, the percentage of intension increased for chloroform extract. For retention time 11.5min, the percentage of intension decreased for extract when compared to chloroform extract.

CONCLUSION

The sample *Ruellia Prostrata*, collected from Kolli hills, was studied in detail and biochemical characterization of the protein isolated from sample was done. Chloroform 50g of sample yielded a total amount of 11.5sg respectively. Crude protein from the sample was extracted at a concentration of 1.8 mg/ml in chloroform extract. The partial purification of protein was done using DEAE cellulose. The chloroform and aqueous extracts showed highest antioxidant activity in DPPH (96.56 and 8.57 %).SDS-PAGE of the crude protein yielded three well defined bands at 109.9, 28.2, 12.4KDa in both the extracts.

REFERENCE

1. Andhiwal CK,Has C, Varshney RP. Antifertility screening and phytochemical investigation of ruellia prostrate poir.j Indian chem. Soc, 1986; 63: 934.
2. Benerjee AK. Sterols from Ruellia prostrate. Current sci (banerjee). American Chemical Society, Washington, DC, 1984; 53: 144-5. 547 2–9.
3. Braca, A., C. Sortino, M. Politi,I.Morelli and J.Mendez.Antioxidant activity of flavanoids from Licania licaniaeflora. Journal of Ethanological Pharmacology, 2002; 79: 379-381.
4. Buccolo G, etal Quantitative determination of serum triglycerides by the use of enzyme clin chem, 1973.
5. Chen, Z. Y. and P. T. Chan. Antioxidative activity of green tea catechins in canola oil. Chemistry and physics of Lipids, 1996; 82: 163-172.
6. Harborne JB. Phytochemical methods. 3rd. Chapman and Hall, London, 1998; 1-257.
7. Ho, C.T., Ferraro, T., Chen, Q., Rosen, R.T., Phytochemical inteas and rosemary and their cancer-preventive properties. In: Ho, C.T.Osawa, T., Huang, M.T., Rosen, R.T. (Eds.), Food Phytochemicals for Cancer Prevention. II. Tea, Spices and Herbs. ACS Symposium Series 547, American Chemical Society, Washington, DC, 1994; 2–9.
8. Ho, C.T., Ferraro, T., Chen, Q., Rosen, R.T., 1994. Phytochemicals.
9. Kong, J.M., L. S. Chia, N.K. Goh, T. F. Chia and R. Brouillard. Analysis and biological activites of anthocyanins. Phytochemistry, 2003; 64: 923-933.

10. Marchand, L, L. Cancer preventive effects of flavanoids a review. Biomedical and pharmacotherapy, 2002; 56: 296-301.
11. Miranda,M.A., de souse, R.R., Sousa, A.C., Querioz, K.C., Augusto, A. C.,Ferreira, C.V and Aoyama,H, phosphoprotein levels, MAPK activites and NF kappa B expression are affected by Fisetin, Molecules, 2009; 439-444.
12. Nakatani, N., S. Kayano,H. Kikuzaki,K.Sumino, K.Katagiri and T. Mitani. Identification, quantitative determination and antioxidative activites of chlorogenic acid isomers in Prune sJournal of Agricultural and Food Chemistry, 2000; 48; 5512-5516.
13. Re, R.,N. Pellegrini, A.Proreggente, A. Pannala, M.Yang and C. Rice-Evans Antioxidant activity applying an improved radical cation decolorization assay, 1999.
14. Rice-Evans, C.A., N. J. Miller and G.Paganga. Structure-antioxidant activity relationships of flavanoids and phenolic acids. Free Radical and Biological Medicine, 1996; 20: 933-956.
15. Rice-Evans, C.A., N. J. Miller and G.Paganga. Structure-antioxidant activity relationships of flavanoids and phenolic acids. Free Radical and Biological Medicine, 1996; 20: 933-956.
16. Subramanian SS, Nair AGR Apigenin glucosides from Thunbergia fragrans and Ruellia tuberosa. Current Scir, 1974; 43: 480-2.
17. Twari RD, Dave P.D-3-3 Isolation and study of the Mucilage from the Seeds of Dipteracanthus prostrates NEES (Carbohydrates). International Symposium on the chemistry of natural products, 1998: 1-257.
18. Velioglu, Y.S., G. Mazza, L Goa and B. D. Oomah. Antioxidant activity and totel phenolics in selected fruits, vegetables, and grain products.Journal of Agricultural and Food chemistry, 1998; 46: 4113-4117.