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Screening of Antioxidant and Proton potassium ATPase inhibitory activity of edible flower extracts

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ABSTRACT: In the present study, flower extracts of plants Pomegranate (Punica granatum) and Coriander (Coriandrum sativum) flowers were screened for their antioxidant activity and proton potassium ATPase inhibitory activity. Among various mechanisms involved in the formation of gastric ulcers, free radicals generated during stress are one of the major causative factors for the gastric lesion through oxidative damage. Thus, the best approach to control gastric ulcer would be to inhibit oxidative damage and acid secretion induced by the enzyme. The proton potassium ATPase inhibitory and antioxidant activity of aqueous and ethanol extracts of two different flower extracts were investigated. The flower extracts were subjected to radical scavenging activity, reducing property, inhibition of lipid peroxidation and acid secretion. Among the two different flower extracts screened, the aqueous extract of Punica granatum was found to contain high amount of phenolics and flavonoids with high antioxidant activity and maximum Proton potassium ATPase enzyme inhibition.

Key words: Punica granatum; Coriandrum sativum; Gastritis; Antioxidant; Proton potassium ATPase

I. INTRODUCTION

Gastritis is the inflammation of the lining of the stomach. The stomach normally secretes acid that is essential in the digestive process. This acid helps in breaking down the food during digestion. When there is excess production of acid by the gastric glands of the stomach, it results in the condition known as acidity. Acidity is responsible for symptoms like dyspepsia, heartburn and the formation of gastric ulcers. The mechanism of free radical mediated damage during ulcer involves lipid peroxidation, which destroys cell membranes with the release of intracellular components such as lysosomal enzymes, leading to further tissue damage [1]. The radicals also promote mucosal damage by causing degradation of the epithelial basement membrane components, complete alteration of the cell metabolism and DNA damage [2]. Ingested food can generate $O_2^{\cdot-}$ and H_2O_2 in the GI tract [3]. Trans fatty acids in processed foods also generate ROS [4]. Despite the protective barrier provided by the mucosa, ingested materials and microbial pathogens can induce oxidative injury and GI inflammatory responses involving the epithelium and immune/inflammatory cells [5]. A modest approach to prevent ulceration is through enhancement of antioxidants, gastric mucin synthesis, scavenging of reactive oxygen species (ROS), inhibition of H^+, K^+-ATPase and Helicobacter pylori growth in the stomach. Although the anti-secretory drugs, such as proton pump inhibitors (PPIs)-omeprazole, pantoprazole etc. and histamine H2-receptor blockers-ranitidine, famotidine etc. are being used to control acid secretion and acid related disorders. Long term use of these PPIs has potential adverse effects. Therefore, the Proton pump inhibitor (PPI) therapy is thought to primarily protect gastric mucosa by inhibiting gastric acid secretion and antioxidant molecules play a significant role in protecting membranes from oxidative damage. The aim of this study was to evaluate the antioxidant activity and proton potassium ATPase inhibitory property of aqueous and ethanol extract of Punica granatum and Coriandrum sativumedible flowers.

II. MATERIALS AND METHODS

The fresh flowers were obtained locally and authenticated by the Department of Botany. Unless otherwise stated all chemicals used in these experiments were of analytical grade, obtained from SRL and Himedia chemicals.

Preparation of leaf extracts

Pomegranate and Coriander flowers were cleaned and washed with distilled water. 1g of samplewas taken and ground with 10ml of distilled water and 10ml ethanol using pestle and mortar and centrifuged at 10,000xg for 10 minutes at 4°C in a cold centrifuge and filtered through Whatmann No.1 filter paper. The aqueous and organic solvent

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extracts were evaporated to drynesss. The yield was calculated and expressed in percentage. The extracts were dissolved at 1mg/ml concentration for performing various activities.

Determination of total phenolics by Folin-Ciocalteau assay

The concentration of total phenolics in the aqueous and ethanolic floral extracts was determined by the Folin-Ciocalteu assay. It involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, its intensity at 725 nm increases linearly with the concentration of phenolics in the reaction medium [6]. In this study Gallic acid was used as spectrophotometric standard. The phenolic contents of the extracts were determined from calibration curve and were expressed in mg of Gallic acid equivalents/ g sample.

Estimation of total flavonoids

Aluminium chloride colorimetric method [7] was used for flavonoids determination in aqueous and ethanolic flower extracts of Punica granatum and Coriandrum sativum. The absorbance of the reaction mixture was measured at 420 nm with UV visible spectrophotometer. The content was determined from extrapolation of calibration curve of Quercetin. The concentration of flavonoid was expressed in terms of mg / g of sample.

Estimation of total sugars

Total soluble sugars were estimated according to the procedure of phenol-sulphuric acid method [8]. To suitable extract, 1ml of phenol and 5ml of conc. H_2SO_4 was added and incubated for 30 mins. The absorbance was measured at 470nm in spectrophotometer. Total soluble sugars were expressed in terms mg of glucose/g of sample. Glucose (0-25micro g) was used as reference standard.

Estimation of protein

Protein content was determined in the aqueous sample extract by the method of Lowry et al., [9] using BSA as protein standard.

Antioxidant activity by DPPH method

Determination of antioxidant activity by the DPPH method [10] was done for all the aqueous and ethanolic flower extracts. Diphenyl picryl hydrazyl ($DPPH^{\cdot}$) was used as a stable radical for assessing antioxidant activity. Reduction of DPPH by an antioxidant result in a loss of absorption at 517 nm. Thus, the degree of discoloration of the solution indicates the scavenging efficiency of the added substances. Percentage of radical scavenging activity was calculated for all the extracts.

Determination of reducing power

The reducing power activity of aqueous and ethanolic flower extracts of Punica granatum and Coriandrum sativum were evaluated according to the method of Yen and Chen [11]. Compounds which have reductones converts potassium ferricyanide to ferrocyanide, which then react with ferric chloride to form ferric ferrous complex which can be measured at 700nm. Increase in absorbance of the reaction mixture indicated the reducing power of the samples.

Antioxidant activity by TBA method

Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) to form a diadduct, a pink chromogen, which can be detected spectrophotometrically at 532 nm as per Halliwell and Gutteridge [12]. The egg yolk was used for the study of invitro lipid peroxidation. The percentage of anti-lipid peroxidative activity (%ALP) per gram of sample is calculated for the aqueous and ethanolic flower extracts.

Inhibition of proton potassium ATPase activity
Preparation of H^+K^+ATPaseenzyme

To prepare H^+, K^+-ATPase enzyme sample, sheep stomach was cut opened, the mucosa at gastric fundus was cut-off, and the inner layer was scraped out for parietal cells [13]. Thus, obtained cells were homogenized in 16 mM Tris buffer (pH 7.4) containing 10% Triton X-100 and centrifuged at 6000 g for 10 min. The supernatant (enzyme extract) was used to determine the H^+K^+-ATPase inhibition.

Assesment of inhibition of proton potassium ATPase activity

The reaction mixture containing 0.1 ml of enzyme extract and different extracts of flowers at 100 µg concentrations was pre incubated for 60 min at 37°C. The reaction was initiated by adding substrate 2 mM ATP (200 µL), in addition to this 2 mM $MgCl_2$ (200 µL) and 10 mM KCl (200 µL) was added. After 30 min of incubation at 37°C, the reaction was stopped by the addition of assay mixture containing 4.5 % ammonium molybdate and 60% perchloric acid followed by centrifugation at 2000 g for 10 min and inorganic phosphate

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released was measured spectrophotometrically at 660 nm by following Fiske-Subbarow method [14]. Briefly, to the 1 ml of supernatant 4 ml of millipore water, 1 ml of 2.5% ammonium molybdate, 0.4 ml of ANSA was added and allowed to stand for 10 min at room temperature. Absorbance of released inorganic phosphate was measured at 660 nm. Enzyme activity was calculated as micromoles of Pi released per gram of sample.

Percentage of enzyme inhibition was calculated by using the formula:

Percentage of inhibition= [Activity $_{(control)}$ − Activity $_{(test)}$/Activity $_{(control)}$] × 100.

III. RESULTS AND DISCUSSION

In recent days emphasis has been on the use of edible sources for maintenance of the health. Fruits, vegetables and other parts of plants are considered as one of the antioxidants rich sources for combating free radical mediated diseases. Flowers are not new to the plate. In many forms, they are routinely consumed, whether as vegetables (cauliflower and banana inflorescence) or as colouring and flavouring agents (rose, saffron, jasmine) and even as spices (cloves are dried buds). The original rose jam—gulkand—has been around for 700 years. Gastritis is the inflammation of gastric mucosa. It may be acute or chronic. Gastric mucosal damage due to the hypersecretion of gastric acid through H^+/K^+-ATPase action is used as one of the strategies in the management of hyperacidity by inhibiting proton potassium ATPase thereby blocking Proton secretion. Although several synthetic proton pump inhibitors (PPIs) such as omeprazole, pantoprazole, lansoprazole, rabeprazole, and esomeprazole are available for use to manage hyperacidity in individuals with gastrointestinal disorders, most of these drugs come with several adverse effects [15,16] which are not tolerated by some individual. In the present study inhibitory and antioxidant activity of aqueous and ethanolic extracts of four different flowers were investigated. Recently peptic ulcer has become a common global problem because of change in life style. Among various mechanisms involved in the formation of gastric ulcers, free radicals generated during stress are one of the major causative factors for the gastric lesion through oxidative damage. Thus, the best approach to control gastric ulcer would be to inhibit oxidative damage and acid secretion induced by the enzyme proton potassium ATPase. The present study therefore was focused on evaluation of inhibition of proton potassium ATPase, one of the causative factor for development of gastric ulcers and screening antioxidant activity to inhibit oxidative damage mediated gastric ulcers by the flower extracts which contained phenolics and other plant secondary metabolites that serve as good antioxidants.

The percentage of yield, protein, carbohydrate and phytochemicals, antioxidant activity and inhibition of proton potassium ATPase activity of Punica granatum and Coriandrum sativum aqueous and ethanolic flower extracts is depicted in Table.1 The total yield of crude extracts from edible flowers of pomegranate and coriander by using the solvents aqueous and ethanol were 15%, 11%, 5% and 4.4% respectively, with reference to the fresh plant material. The yield was found to be maximum in aqueous extract of Pomegranate flower.

Total Phenolics was estimated for all the four extracts at 100 µg concentration as shown in Figure 1 and Table 1. Out of the four extracts, aqueous Punica granatum flower extract with 275 mg gallic acid equivalent/g of sample showed maximum amount of phenolics followed by other extracts. The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties. These results give a reason for the activity of these plants as antioxidant and how these plants extracts enable to scavenge the free radicals. Despite their wide distribution, the health effects of dietary polyphenols have come to the attention of nutritionists only in recent years. Researchers and food manufacturers have become more interested in polyphenols due to their potent antioxidant properties, their abundance in the diet, and their credible effects in the prevention of various oxidative stress associated diseases.

Flavonoids are important secondary plant metabolites, which increase with plant stress. The total flavonoid content of edible flowers of aqueous and ethanol extracts of Punica granatum and Coriandrum sativumis found to be 1760, 900, 300, 132mg/g of sample respectively as depicted in figure 2 and Table 1. Aqueous Punica granatum flower extract was found to contain high amount of flavonoids and least being in ethanol Coriandrum sativumflower extract.

The aqueous and ethanol extracts of two different flowers, Punica granatum and Coriandrum sativum were screened for the total reducing sugars and aqueous extracts for protein concentration at 100 µg concentration of extracts. These serve as the primary metabolites important for their sustainable development and that also indicates the nutritional aspects of different flowers. The results are

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depicted in the figures 3 and 4 and table 1. The aqueous extract of Punica granatum flower ranked the highest in total sugars and protein with 30.25mg and 2420mg/ g of sample.

ROS like superoxide radical anion and hydroxyl radical are now considered one of the major causative factors for mucosal lesions through oxidative stress. The radicals promote mucosal damage by causing degradation of epithelial basement components, complete alteration of the cell metabolism and DNA damage [17]. Free radical scavenging potentials of all the four edible flower extracts at 100 µg concentrations were tested by the DPPH method and the results are shown in figure 5. The aqueous Punica granatum flower extract showed maximum free radical scavenging activity of 99.53% followed by other extracts. The activity of extracts is attributed to their hydrogen donating ability. Increasing the number of hydroxyl or catechol groups increases radical scavenging activity. In presence of other H-donating groups (sulfhydryl, amide) in molecule also accelerates this activity.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Figure 6 shows the reducing power activity of different edible flower extracts at 100 µg concentration using the potassium ferricyanide reduction method. Thus, the good reducing activity was observed in aqueous Punica granatum flower extract. The reducing power activity is due to the presence of reductones (Phenolics) which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation.

Lipids and proteins are more susceptible to oxidative damage. The mechanism of free radical mediated damage during ulcer involves lipid peroxidation, which destroys cell membranes with the release of intracellular components, such as lysosomal enzymes, leading to further tissue damage. The results of the effect of various edible flower extracts at 100 µg concentration to prevent lipid peroxidation are shown in figure 7. At 100 µg concentration, the edible flowers of aqueous and ethanol extracts of Punica granatum and Coriandrum sativum showed 82.51%, 24.13%, 31.25% and 37.5% of inhibition of lipid peroxide generation with highest seen in Punica granatum Aqueous flower extract.

Proton pump inhibitors (PPIs) are widely prescribed to protect against gastric ulcer. The aqueous Punica granatum flower extract showed significant proton pump inhibitory activity of 90.9% as depicted in figure 8. This study was carried out to evaluate the proton potassium ATPase inhibitory activity of different flowers. Inactivation of the enzyme can be the major gastroprotective mechanism.

IV. CONCLUSION

Therefore, aqueous Punica granatum flower extract with high concentration of phenolics, flavonoids and high antioxidant activity possess maximum enzyme inhibition. Further the purification and isolation of the active ingredient in aqueous Punica granatum flower extract is to be studied for the therapeutic value.

V. ACNOWLEDGEMENT

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Table 1: Biochemical, phytochemical and antioxidant activity of aqueous and ethanol edible flower extracts.

Sl no	Name of the Plant	Yeild %	Total Phenolics (mg/g of Sample)	Total Flavanoids (mg/g of Sample)	Total Sugars (mg/g of Sample)	Protein (mg/g Of Sample)	DPPH Assay (% radical Scavenging Activity)	Reducing Power Assay (OD at 700nm)	TBA assay (Anti lipid Peroxidation)	Inhibition Of Proton Potassium ATPase Assay
1	Punica Granatum	15	275	1760	30.25	2420	99.53	1.58	82.51	90.9

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	(aqueous)									
2	Punica Granatum (Ethanol)	11	187.5	900	18.75	-----	92	0.78	24.13	40.3
3	Coriandrum Sativum (aqueous)	5.0	26	300	10.75	1200	74.9	0.63	31.25	79.6
4	Coriandrum Sativum (Ethanol)	4.4	22.88	132	4.4	----	83.628	0.15	37.5	54.4

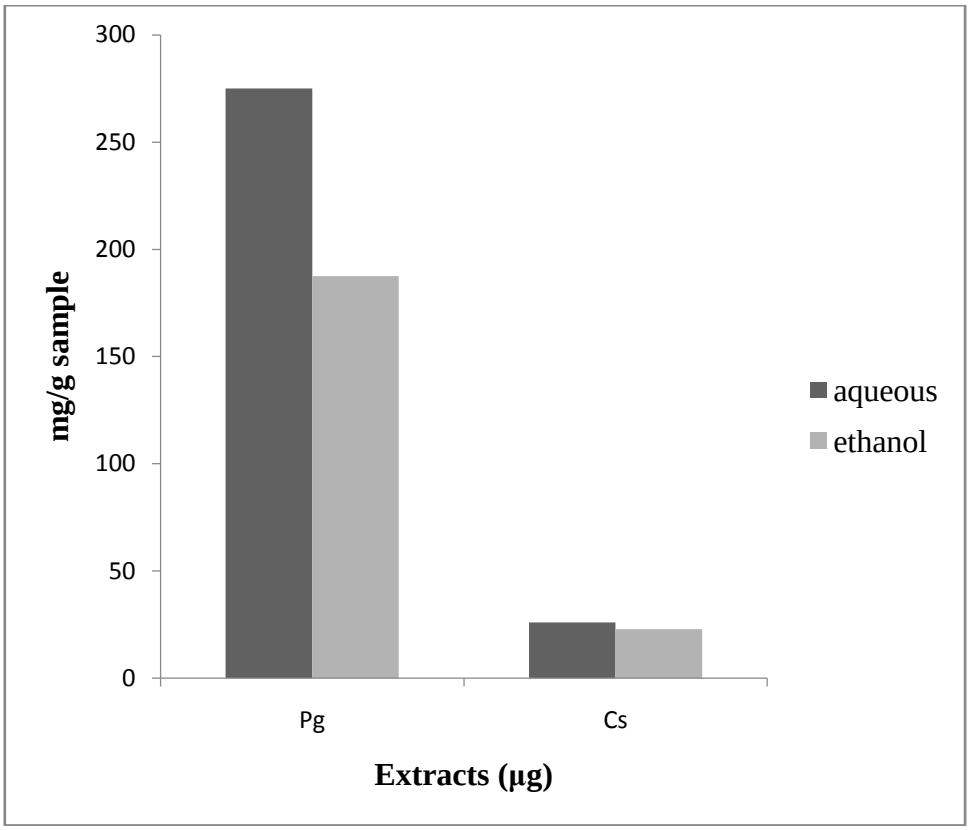

Figure 1: Total phenolics in edible flower extracts.Pg (Punica granatum), Cs (Coriandrum sativum)

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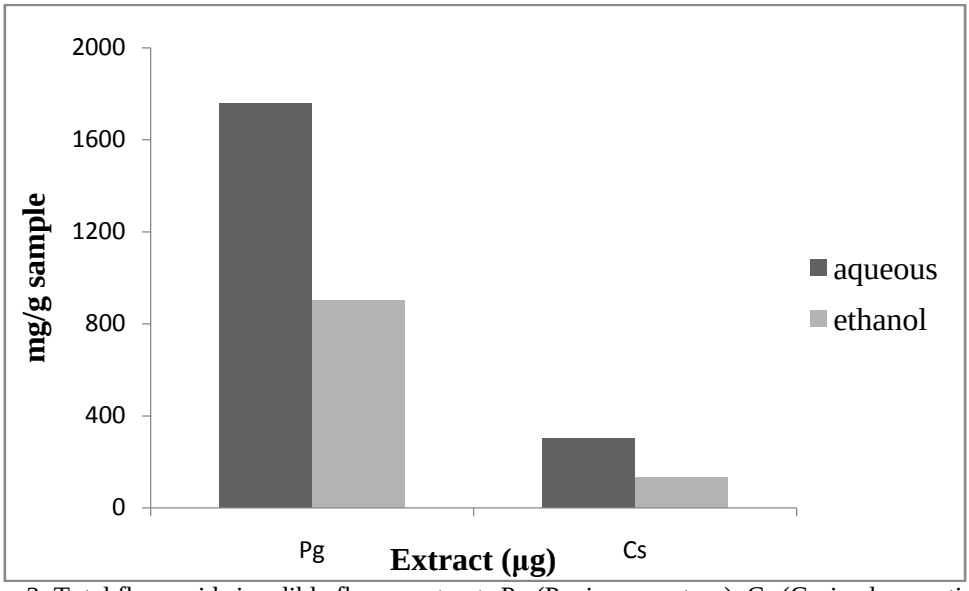

Figure 2: Total flavonoids in edible flower extracts.Pg (Punica granatum), Cs (Coriandrum sativum)

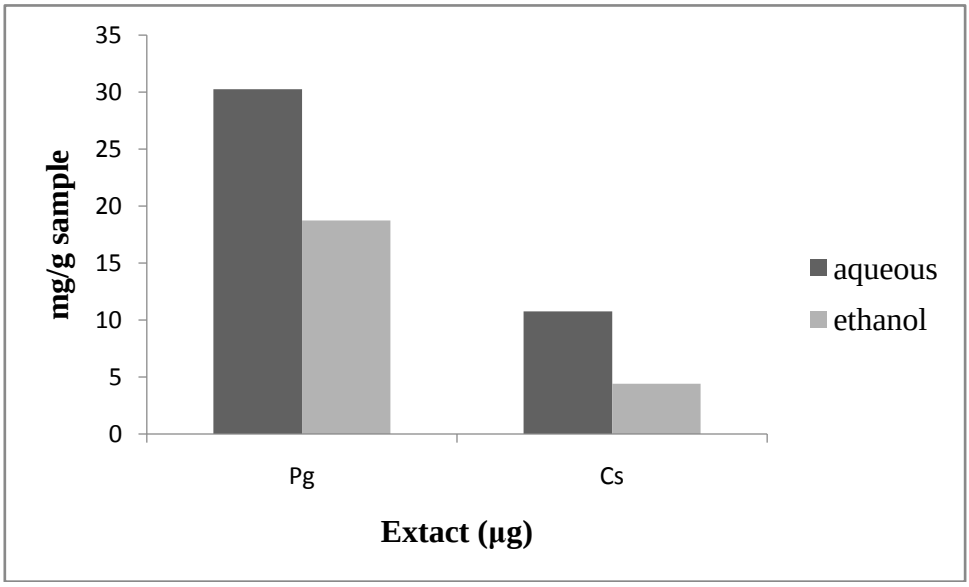

Figure3: Total sugarsin edible flower extract.Pg (Punica granatum), Cs (Coriandrum sativum)

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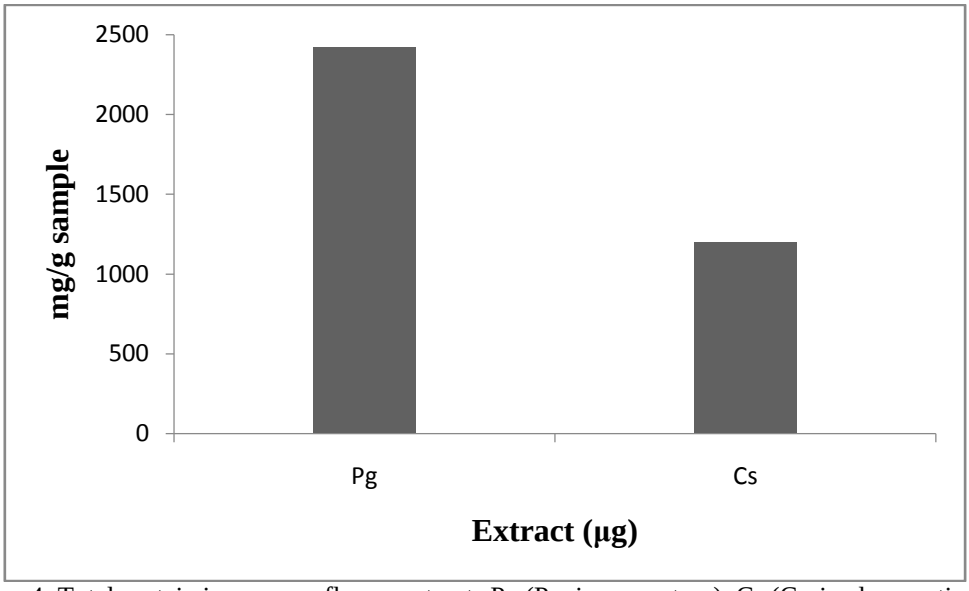

Figure4: Total protein in aqueous flower extracts.Pg (Punica granatum), Cs (Coriandrum sativum)

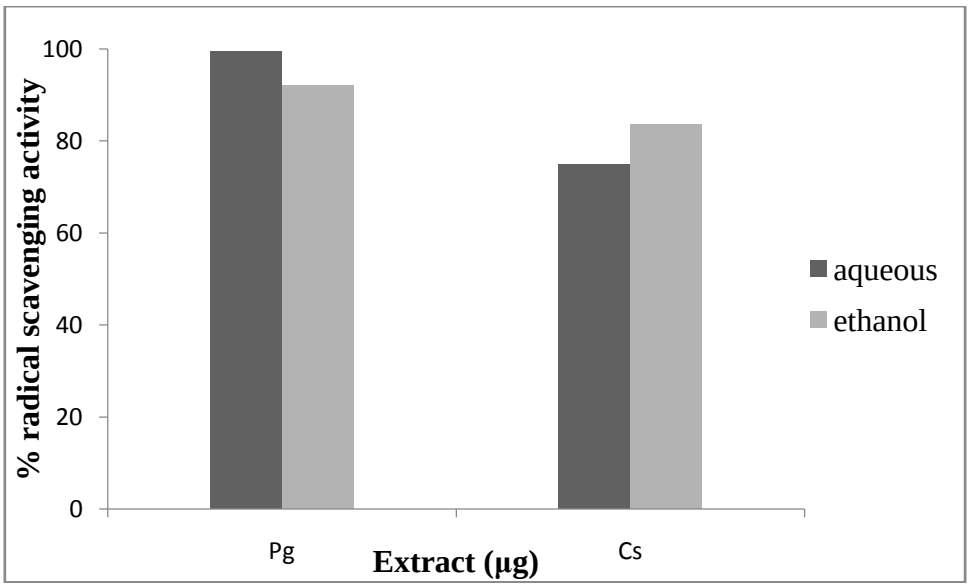

Figure 5: Percentage of radical scavenging activity by edible flower extracts. Pg (Punica granatum), Cs (Coriandrum sativum)

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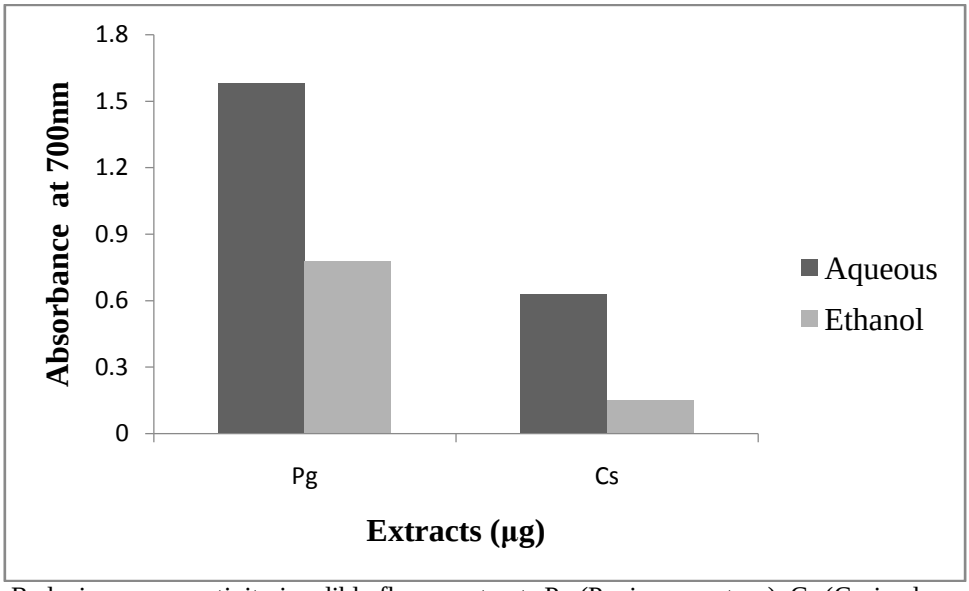

Figure 6: Reducing power activity in edible flower extracts.Pg (Punica granatum), Cs (Coriandrum sativum)

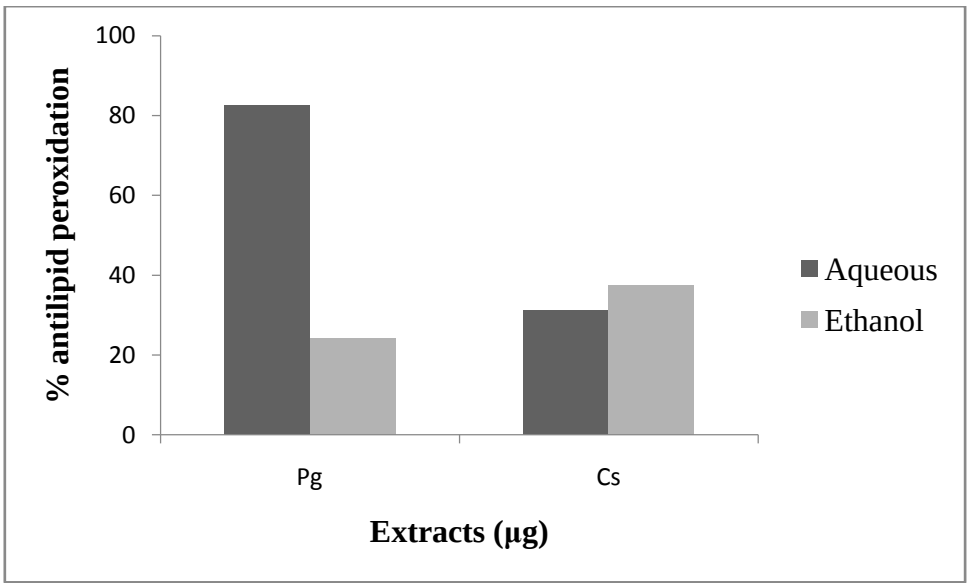

Figure 7: Percentage of anti- lipid per oxidation activity in edible flower.Pg (Punica granatum), Cs (Coriandrum sativum)

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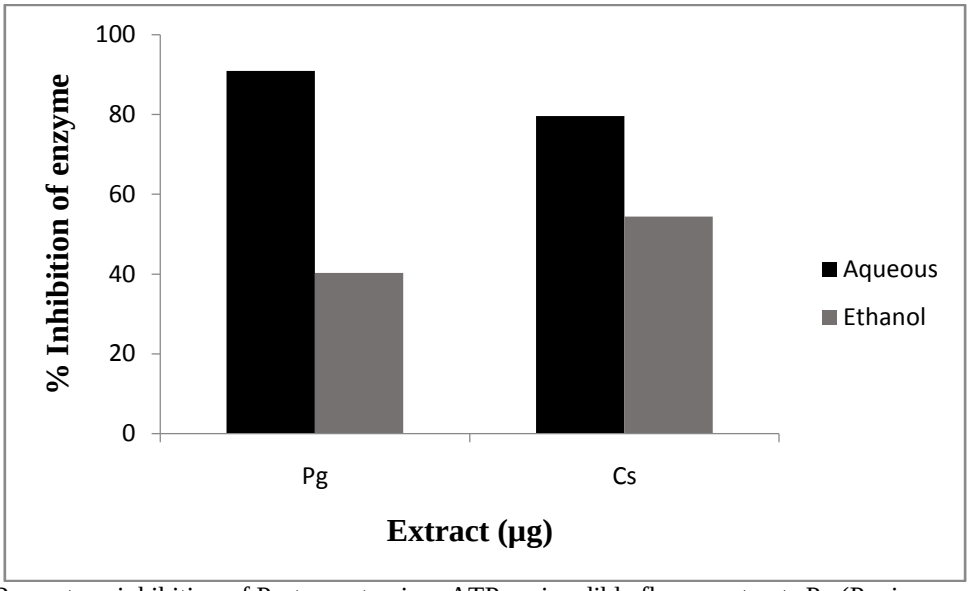

Figure 8: Percentage inhibition of Proton potassium ATPase in edible flower extracts.Pg (Punica granatum), Cs (Coriandrum sativum)

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