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Research Article

ANTIULCEROGENIC AND FREE RADICAL SCAVENGING ACTIVITY OF FLAVONOID FRACTION OF *PSIDIUM GUAJAVA* LINN LEAVES

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ABSTRACT

The incidence of peptic ulcer has reached a major health problem with multifactorial etiology. Culturally acceptable treatment options are limited for ulcer complications resulting in gastric mucosal damage. Here, we identified and described the botanicals capable of protecting ulcer by chemical and physical induced method. The enhancement of the gastric mucus has been proposed to explain the antiulcer activity of different class of flavonoid. So in the present study flavonoid fraction was isolated from *Psidium guajava* leaves *(Myrtaceae)* and tested for antiulcer activity. The flavonoid in alcoholic extract (EEPG) and its ethyl acetate fraction (EAFPG) were identified by Thin Layer Chromatography and estimated by aluminium chloride method. Antiulcer activity of ethanolic extract of *Psidium guajava* (EEPG) and its flavonoid fraction (EAFPG) were studied in rats by ethanol induced and pylorus ligature model at the dose level of 200mg/kg orally 30minutes prior to ulcer induction. The antiulcer activity was assessed by determining and comparing the ulcer index in the test group with that of vehicle control group. Ranitidine was used as a reference standard drug. The free radical scavenging activity of EEPG and EAFPG were also evaluated by DPPH and Nitric oxide radical inhibition assays and compared with standard. The ulcer index in the EAFPG treated group was found to be significantly more compared to vehicle control. The significant antiulcer activity of EAFPG was however may be due to the presence of flavonoids which gave the highest gastric protection. Our results suggest that EAFPG possess significant antiulcer activity and free radical scavenging property which could be due to presence of flavonoid. Since, most of the bioflavonoid has the ability to show the gastric protector effects by inhibition free radicals.

Keywords: Antiulcer; DPPH; Flavonoid; Free radicals; Nitric oxide; *Psidium guajava.*

INTRODUCTION

Recently there has been a rapid progress in understanding the pathogenesis of peptic ulcer. These have been made possible largely by the availability of the proton pump inhibitors, histamine receptors blockers, drug affecting the mucosal barrier and prostaglandin synthesis (Parmar NS *et al*., 2003). However the clinical evaluation of these synthetic drugs showed development of tolerance and incidence of relapses and side effects make their efficacy arguable. Drugs of plant origin are gaining popularity and are being investigated for a number of gastric disorders including peptic ulcer. Indian medicinal plants and their derivatives have been used as an invaluable source of therapeutic agents to treat various gastric disorders. Flavonoids have a broad scale of biological activities among which antiulcer effect stands out. The enhancement of the gastric mucus has been proposed to explain the antiulcer activity of different class of flavonoid (Martin M J *et al*., 1998).

Psidium guajava Linn. belongs to *Myrtaceae,* is a wide spread plant in India and commonly used as antiseptic, anthelmintic, wound healing and in ulcers (Khanikar G, 2005). It has a high content of flavonoid (Quercetin) reviewed from literature (Abdelrahim S I *et al*., 2002). The young leaves are used as tonic in the diseases of the digestive function and is said to be remedy for toothache (Asima chattrerji *et al*., 1992). Numerous flavonoids like genistein, sophoradin, quercetin, naringenin and silymarin have shown antiulcer properties in different experimental models (Alarcon de lastra C *et al*., 1994). Literature review indicated that the ulcer protective activity of flavonoid rich fraction leaf extract of the plant has not been evaluated so far. Thus the aim of the study was to evaluate protective effect flavonoid fraction of *Psidium guajava* in ulcer induced by chemical and physical methods on experimental animal. **(**Rakesh K Sindhu et al., 2010)

MATERIALS & METHODS

Plant material

The Plant specimen (leaves) for the proposed study was collected during the month of September 2009 from Vel's University herbal garden, Pallavaram, Chennai. It was identified and authenticated By Dr. P. Jayaraman, Director, Plant Anatomy Research centre (PARC), Chennai, Tamil Nadu.

Animals

Wistar albino rats (150 ± 200gm) were used for the present study. Animals were housed in polypropylene cages maintained under standard laboratory conditions 12:12 hour's light and dark sequence at an ambient temperature of 25 ± 2^{0}C; 35-60% humidity. The animals were fed with standard rat pellet diet and water *ad libitum*. The experimental protocol of pharmacological and toxicological studies were reviewed and approved by the Institutional Animal Ethics Committee (IAEC). (290/CPCSEA/ 11-03-2010).

Extraction

The leaves of *Psidium guajava* L. were shade dried and coarsely powdered. About 300gms powdered leaf drug was extracted with ethanol 90%v/v by cold maceration method for 72 hours. It was filtered and concentrated by distilling off the solvent and evaporated to dryness under reduced pressure to yield Ethanolic Extract of *Psidium guajava* (EEPG). Then it was extracted successively with solvents of increasing polarity such as Hexane, chloroform and ethyl acetate to yield its respective fraction. All the fractions were evaporated under vacuum. Its colour and consistency were observed. The percentage yield was calculated on the air-dried basis.

Phytochemical screening

The alcoholic extract and its fractions (Hexane, chloroform and ethyl acetate) were subjected to Shinoda and sodium hydroxide test for identification of flavonoid. Among the tested fraction Ethyl Acetate Fraction of *Psidium guajava* (EAFPG) showed positive answer for the above test. So the total ethanolic extract and its ethyl acetate fraction were further identified by Thin Layer Chromatography.

Acute toxicity study

The acute oral toxicity study was carried out as per the OECD guidelines-423. Animals were observed individually after administration of *EEPG* and *EAFPG,* during the first 30 minutes, and

periodically 24 hours with special attention given during the first 4 hours and daily thereafter for a total of 14 days for toxic symptoms and mortality. All observations are systematically recorded with individual records being maintained for each animal. From the report it was found that the extract was not toxic up to 2000 mg/kg dose level. One-tenth dose of the maximum dose used in the acute toxicity study was considered as therapeutic dose for further pharmacological study. (OECD Test Guideline-423., 2001)

Preparation of standard and test samples

Standard drug Ranitidine (20mg/kg body weight) and the test sample *EAFPG* (200mg /kg per body weight) and *EEPG* (200mg/kg per body weight) were suspended in 2 % w/v carboxy methyl cellulose (CMC) using distilled water.

Chemicals and drugs

All standard drugs were obtained from Cipla, Mumbai, and chemicals for the present study were purchased from Sigma chemicals, Chennai.

Thin Layer Chromatography

Preparation of test and standard sample

10mg of ethanolic extract (test sample) and its ethyl acetate fraction were dissolved separately in 5ml of methanol. 1µl was applied as a spot. 10mg of quercetin (standard sample) was dissolved in 5ml of methanol. 1µl was applied as a spot.

Developing solvent system

A number of developing solvent systems were tried. But the satisfactory resolution was obtained in Toluene: Ethyl acetate: Formic acid (6: 3:1). After development of plate, it was air-dried and numbers of spots were noted and R_f Values were calculated. Spots were visualized by spraying with sodium hydroxide as detecting agent.

Estimation of flavonoid (Kadifkova P *et al*., 2005)

Total flavonoid content in dried ethanolic and its ethyl acetate fraction of aerial parts of the plant were estimated by spectrometric method [Perkin-Elmer UV spectrometer Lambda 16 (Germany)]. Dried powdered plant material (10gms) was extracted by continuous mixing in 100ml of 70% v/v ethanol for 24 hours at room temperature. After filtration, ethanol was evaporated until only water remained. Water phase was subsequently extracted with ethyl acetate. The extract was dried over anhydrous sodium sulphate, filtered and concentrated under vacuum up to a concentration of 1gm/ml of extract. They were further diluted with ethyl acetate to obtain 0.01 gm/ml solutions. About 10 ml of the solution was transferred into a 25 ml volumetric flask, 1 ml of 2% $AlCl_3$ was added and the solution was filled to volume with methanol-acetic acid and was kept aside for 30 min. The absorbance was measured at 390 nm against the blank. Quercetin was used as reference to construct the calibration curve in the concentration range of 10.0-100.0 µg/ml.

Free radical scavenging activity

Nitric oxide scavenging assay

Nitric oxide scavenging assay was measured by spectrophotometric method (Amarowicz R *et al*., 2008). Sodium nitroprusside (5mM) in phosphate buffer saline was mixed with different concentrations (50-1000 µg/ml) of ethanol extract and its ethyl acetate fraction of leaves *Psidium guajava* sample were dissolved in ethanol and ethyl acetate incubated at 25ºC for 30 min. After 30 min 1.5 ml of the incubation solution was removed and diluted with 1.5 ml of Griess reagent (1% sulphanilic acid, 2% Phosphoric acid and 0.1% N-1-napthylethylene diamine dihydrochloride). The absorbance was measured at 546 nm. All determination was performed in triplicate. The percentage of Nitric oxide radical scavenging ability of the sample was calculated from the absorbance value at the end of 15 min duration as follows. The IC_{50} Values for extracts were also calculated.

$$\% \text{ Inhibition} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}})}{(\text{Abs}_{\text{Control}})} \times 100$$

Where Abs- control is absorbance of control, Abs-sample is absorbance of test sample.

DPPH radical Assay (Maryam Zahin et al., 2009)

The effect of ethanolic extracts its ethyl acetate fraction of leaves *Psidium guajava* were estimated by DPPH radical assay (Rajkumar DV *et al*., 1993). 2 mL of DPPH in methanol was added to various concentrations (50-1000 µg/ml) of test substance. The mixture was shaken for 15 seconds and left to stand at 37^{0}C for 30 min. The decrease in the absorbance at 515nm was continuously recorded in a spectrophotometer for 15 min at room temperature. All determination was performed in triplicate.

The DPPH scavenging activity (decrease of absorbance at 515 nm) of extract was plotted against time and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 15 min duration as follows. The IC_{50} Values for test extracts were also calculated**.**

$$\% \text{ Inhibition} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}})}{\text{Abs}_{\text{Control}}} \times 100$$

Where $\text{Abs}_{\text{Control}}$ is absorbance of control at time = 0, $\text{Abs}_{\text{Sample}}$ is absorbance of test sample at time = 15 min.

Antiulcerogenic activity

Pylorus ligation induced ulcer model (Sairam K *et al*., 2001)

Wistar rats (150-200 gms) of either sex were fasted for 18 hours but water *ad libitum*. Just one hour before the experiment, water was withdrawn. Under light ether anaesthesia the abdomen was opened by a midline incision below the sternum. Pylorus was lifted and ligated carefully with the help of silk thread, without damaging blood vessels. The stomach was replaced carefully and abdomen was then sutured. Both standard and test sample were administered to the animals by orally. The animals were deprived of food and water after pylorus ligation, and sacrificed by decapitation at the end of 4 hour. The abdomen was opened at the esophageal end. The entire stomach was dissected out and a small cut was given to the pylorus region just above the knot and the gastric contents were collected in the graduated centrifuge tubes. Gastric volume, pH free acidity and total acidity were measured. The stomach was cut open along the greater curvature and pinned on a soft board for evaluating gastric ulcer and ulcer index.

Drug treatment plan

The rats were fasted for 18 hrs prior to the commencement of the experiment were randomly divided into four groups and treated as follows. Group I Vehicle (5 ml/ kg body weight) of 2% w/v CMC in distilled water. Group II Standard drug Ranitidine (20 mg/kg body weight) suspended in 2% w/v CMC in distilled water. Group III EEPG (200mg/kg orally body weight) in 2% w/v CMC in distilled water. Group IV EAFPG (200mg/kg orally body weight) in 2% w/v CMC in distilled water.

Evaluation of biochemical parameters

Collection of gastric juice

The gastric juice was collected from the pylorus ligated stomach of rats and the contents collected was centrifuged for 30 minutes (3,000 rpm).The gastric juice was subjected to biochemical estimations.

Determination of gastric juice volumes and pH (Deshpande SS *et al*., 2003)

The volume of gastric juice and pH of centrifuged gastric secretions were measured by pipette and pH meter, respectively.

Estimation of Free and Total acidity (Kulkarni S K, 1995)

One ml of supernatant gastric juice was diluted with 9 ml of distilled water. A volume of 2ml diluted gastric juice taken out used a pipetted into a 100 ml conical flask, added 2 or 3 drops of Topfer`s reagent and titrated with 0.1N NaOH until all traces of red colour disappears and the colour of the solution was yellowish orange. This volume corresponds to free acidity. Then 2 or 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge appears. Again the total volume of alkali added was noted. Acidity can be expressed as MEq/l/100g.

Acidity was calculated by using the formula given below-

Acidity = Volume of NaOH × Normality of NaOH × 100 / 0.1 µ/L/100gm

Calculation of gastric ulcer index (Goyal R K *et al*., 2002)

The dissected stomach of the sacrificed rats was opened along the greater curvature and the ulcer index calculated from the glandular portion of the stomach. The ulcer index was calculated as, Ulcer index = 10/x, where x = Total mucosal surface/Total ulcerated area. Each lesion was measured along the greatest length. The total area of the glandular portion of the stomach and that of ulcerated mucosa were measured for determination of the ulcer index. The ulcer score was expressed as ulcer index for each group. The ulcer index was calculated using the formula.

Calculation of percentage protection

$$\text{Percentage protection of Ulcer} = \frac{UI_c - UI_t}{UI_c} \times 100$$

Where UI_t = Ulcer index of treated group, UI_c = Ulcer index of the control group.

Ethanol induced ulcer model (Uma maheswari *et al*., 2007)

The rats were fasted for 18 hrs with free access of water. The gastric ulcers were induced in each animal by administering 80 % Ethanol (1ml/200 gm Body weight). After 1 hrs the animals were sacrificed by cervical dislocation. The stomach was incised along the greater curvature pinned on a soft board examined for gastric lesion in mm. The ulcer index was scored, the percentage inhibition was calculated.

Drug treatment plan

The rats were fasted for 18 hrs prior to the commencement of the experiment were randomly divided into four groups are as follows. Group I Vehicle (5ml/ kg body weight) of 2% w/v CMC in distilled water. Group II Standard drug Ranitidine (20 mg/ kg body weight) suspended in 2% w/v CMC. Group III EEPG (200mg/ kg orally body weight) in 2% w/v CMC in distilled water. Group IV EAFPG (200 mg/ kg orally body weight) in 2% w/v CMC in distilled water.

Calculation of gastric lesions

The gastric lesion were counted, and an ulcerative index (UI) was calculated for each animal as follow: (UI= (n lesion I)+(n lesion II)^2+(n lesion III)^3), where I=Presence of edema, hyperemia and single sub mucosal, hemorrhages. II= Presence of submucosal, hemorrhagic lesions with small erosions. III= Presence of deep ulcer with erosions and invasive lesions.

Statistical analysis

For all the above methods, the results were expressed as mean ±SEM. Statistical analysis was done using one way ANOVA followed by multiple comparison test using the Graph pad prism software.

RESULTS AND DISCUSSION

The successive fractionation of leaves of *Psidium guajava* L was done in the order of increasing polarity i.e. hexane, chloroform and ethyl acetate. The phytochemical test was carried out for the identification of flavonoid. Among the tested fractions EAFPG only answers positively for flavonoid. Flavonoid fraction of ethanolic extract of the plant has not been screened for antiulcer activity. Phytochemical screening was carried out to identify the phytoconstituents present in the ethanol extract and its fraction. Phytochemical screening of ethyl acetate fraction shows the presence of flavonoids. The total flavonoid content was estimated using aluminium chloride by colorimetric method. The results indicates that the content of flavonoid was found to be higher in ethanol extract

Table 1: Total flavonoid content of ethanolic extract and its fraction

S. No.	Extract/Fraction	Values (mg % w/w)
1	Ethanol extract	3.10
2	Ethyl acetate fraction	2.60

Further flavonoid was identified by compared with standard sample (Quercetin) by TLC. It was performed using solvent system Toluene: Ethyl acetate: formic acid (6:3:1 V/V) using sodium hydroxide as detecting agent. The result observed in the ethanolic extract shows different R_f value (0.21, 0.51, 0.78, 0.82) .and standard R_f value (0.82), ethyl acetate fraction R_f value (0.78). TLC was done for the ethanolic extract and its ethyl acetate fraction on support of the chemical test since it showed yellow colour spot with sodium hydroxide, confirmed the presence of flavonoids. It was identified as quercetin by comparing its R_f value with that of standard quercetin.

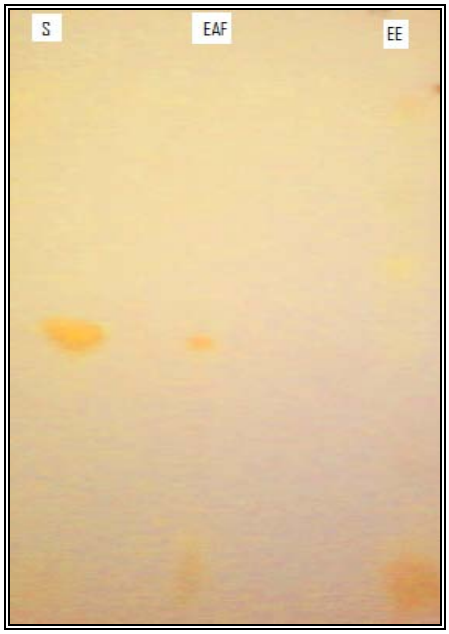

Fig. 1: TLC of Leaf extract of *Psidium Guajava* Linn

S-Standard (Quercetin), EAF-Ethyl acetate fraction, EE-Ethanol extract

Table 2: Thin layer chromatography of *Psidium guajava* Linn Leaves

S. No.	Test extract	Solvent system	Detecting agent	Number of spots	R_f values
1	Std(Quercetin)	T: E: F (6:3:1)	NaoH	1	0.82
2	EAFPG	T: E: F (6:3:1)	NaoH	1	0.78
3	EEPG	T: E: F (6:3:1)	NaoH	4	0.800.780.510.21

R_f - Retardation factor; T: E: F - Toluene: Ethyl acetate: Formic acid; EAFPG-Ethyl acetate fraction leaves of *Psidium guajava* Linn; EEPG-Ethanolic Extract leaves of *Psidium guajava* Linn.

The results of Nitric oxide scavenging activity represents that stable NO radical was effectively scavenged the test extracts. The values of NO scavenging effect (% inhibition) of different concentration of ethanolic extract and its ethyl acetate fraction leaves of *Psidium guajava* (50µg/ml to 1000µg/ml) and Standard (vitamin - E). As the value (% inhibition) is increased, the absorbance decreases with increased concentration of antioxidant. Vitamin -E was used as standard which have greater percentage of inhibition value than extracts. Nitric oxide (NO) is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. Nitric oxide is a very unstable species under aerobic conditions. It reacts with oxygen to produce stable product nitrate and nitrite through intermediates. It was estimated by using Griess reagent and in presence of test compound which was the scavenger. In this study the nitrite produced by the incubation of solutions of sodium nitroprusside in standard phosphate saline buffer at 25 ^{0}C was reduced by the EEPG and EAFPG compared to ethanolic extract ethyl acetate fraction produced a good significant free radical scavenging property which may be due to the presence of flavonoid (quercetin).

The results of DPPH represents that stable DPPH radical was effectively scavenged the test extracts. The values of DPPH scavenging effect (% inhibition) of different concentration of Ethanolic extract and its ethyl acetate fraction of *Psidium guajava* Linn (50µg/ml to 1000µg/ml) and Standard (Ascorbic acid). As the value (% inhibition) are increased, the absorbance decreases with increased concentration of antioxidant. DPPH assay is considered a valid method to evaluate scavenging activity of antioxidants, since the radical compound is stable and does not have to generate as in other radical assays.

DPPH radicals react with suitable reducing agents and then electrons become paired off and the solution loses colour stoichiometrically with the number of electrons taken up. Such reactivity has been widely used to test the ability of plant extract to act as free radical scavengers. DPPH assay of EEPG and EAFPG showed a dose dependent increase in the percentage of inhibition of free radicals. The ethanolic fraction was found to contain more total flavonoids content. It also showed a good antioxidant potential.

Table 3: Nitric oxide scavenging activity of EAFPG, EEPG and Standard

S. No.	Concentration (µg/ml)	% Inhibition		
		STD	EAFPG	EEPG
1	50	8.30±0.45*	5.50±0.21*	2.7±0.31*
2	100	16.66±0.90**	13.80±0.41**	11.11±0.62**
3	200	27.77±1.08**	25.00±0.59**	22.22±0.57**
4	400	52.77±0.60**	50.00±0.63**	44.44±0.84**
5	800	61.11±0.51**	58.33±0.73**	55.55±0.64**
6	1000	65.88±0.84**	63.88±0.83**	61.11±0.74**
7	IC_{50} Value	380 (µg/ml)	400(µg/ml)	620(µg/ml)

EEPG- Ethanol extract of *Psidium guajava*; EAFPG - Ethyl acetate fraction of *Psidium guajava;* Results were expressed as mean ±SEM, Statistical test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6); All values are significant **P<0.01 compared to standard; values are significant *P<0.05 when compared against standard.

Table 4: DPPH radical scavenging activity of EAFPG, EEPG and Standard

S. No.	Concentration (µg/ml)	% Inhibition		
		STD	EAFPG	EEPG
1	50	26.98±0.30**	23.80±0.20*	20.63±0.48*
2	100	34.92±0.42**	31.74±0.49**	28.57±0.70**
3	200	42.85±0.60**	39.68±0.50**	36.50±0.56**
4	400	50.79±0.50**	47.61±0.37**	44.44±0.67**
5	800	58.73±0.76**	55.55±0.28**	52.30±0.76**
6	1000	66.66±0.20**	63.49±0.36**	60.03±0.87**
7	IC_{50} Value	410 (µg/ml)	520µg/ml)	640(µg/ml)

EEPG- Ethanol extract of *Psidium guajava;* EAFPG - Ethyl acetate fraction of *Psidium guajava;* The results were expressed as mean ±SEM, statistical test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6); All values are significant **P<0.01 when compared against standard; All values are significant *P<0.05 when compared against standard

Animals in the Pylorus ligation group showed a significant (**p<0.01) increase in the ulcer index and acid secretary parameters like gastric volume, pH, free and total acidity when compared with those of vehicle treated group .the EEPG (200 mg/kg) and EAFPG (200 mg/kg) treated animal showed ulcer index score value 5.0 and 1.3 which was significantly (**p<0.01) compared to control group value 24. The extract also significantly reduced the gastric volume, total and free acidity and increased the pH of the gastric fluid, proving its antisecretory activity. The EEPG and EAFPG at dose of 200mg/kg showed protection index of 76.7% and 83.7% respectively, whereas standard drug Ranitidine(20 mg/kg body weight) showed protection index of 83.7%.Ulcer has long been recognized as one of the most important gastrointestinal problem. With the ever growing interest in natural medicine, many plants have been screened and reported to be useful in treating and managing ulcer. Peptic ulcer results due to overproduction of gastric acid or decrease in gastric mucosal production. Pylorus ligation induced ulcers occur because of an increase in acid- pepsin accumulation due to pylorus obstruction and subsequent mucosal digestion (Goel R K *et al*., 1991). Further the role of free radicals is also reported in the induction of ulcers.

The present study reveals that EEPG and EAFPG treated groups showed a significant increase in gastric juice pH, reduces the gastric volume, free acidity and total acidity when compared to control. This effect was similar to Ranitidine treated group. Test group decreased the ulcer index more effectively in a dose dependent manner. These result shows that the antiulcer activity of EEPG and EAFPG might be due to its antisecretory activity.

Table 5: Effect of EAFPG and EEPG in Pylorus ligation Ulcer model

Group	Dose	Gastric pH	Gastric volume (ml/100gm)	Free acidity (µ/L)	Total acidity(µ/L)	Ulcer Index	% Protection
Control	2%CMC (5ml/kg)	2.0±0.07	1.4±0.05	28±1.3	45±2.0	24±1.4	-
Std	Ranitidine (20mg/kg)	3.5±0.03	0.6±0.04	24±0.6	40±1.0	3.5±2.0	94.0
Test-1	EEPG(200mg/kg)	3.0±0.08*	0.8±0.03*	25±1.2*	36±1.9*	5.0±2.5*	76.7*
Test-2	EAFPG(200mg/kg)	3.4±0.02**	0.5±0.02**	23±0.9**	38±1.7**	1.3±1.0**	83.7**

EEPG- Ethanol extract of *Psidium guajava;* EAFPG - Ethyl acetate fraction of *Psidium guajava;* Results were expressed as mean ±SEM, Statistical test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6); All values are significant **P<0.01 when compared against control; All values are significant **P<0.01,*p<0.05 compared to standard

Administration of ethanol produce haemorrhagic gastric lesions in the gastric mucosa of the control group. Test extract EEPG (200mg/kg Body weight) and EAFPG (200mg/kg Body weight) reduced these lesions as shown by a significant (**p<0.01) reduction in the ulcer index when compared with the control group. The EEPG (200mg/kg Body weight) and EAFPG (200mg/kg B.W.) showed protection index of 62.7 % and 78.7.4% respectively, Ranitidine also significantly (**p<0.01) reduced ulcer index of ethanol induced gastric ulcer. Ethanol serves as a most common ulcerogenic agent and when given intra-gastrically to rats it produces severe gastric haemorrhagic erosions**.** Ethanol induced gastric ulcers have been widely used for the evaluation of gastro protective activity. Ethanol is metabolized in the body and release super oxide anion and hydroperoxy free radicals. it has been found that oxygen derived free radicals are implicated in the mechanism of acute and chronic ulceration in the gastric mucosa and scavenging these free radicals can play an appreciable role in healing these ulcers. EEPG and EAFPG significantly reduced the ulcer index and significant protection against ethanol induced ulcer. The antioxidant properties of test group may have scavenged the free radicals produced by the metabolism of ethanol and there by heal the ulcers.

Table 6: Effect of EAFPG and EEPG in Ethanol induced Ulcer model

S. No.	Group	Dose	Gastric lesion	Ulcer index	% Protection
1	Control	2% CMC(5ml/kg)	5.62±0.56	15±2.6	-
2	Standard	Ranitidine (20mg/kg)	2.45±0.60	3.2±1.0	89.4
3	Test -1	EEPG(200 mg/kg)	1.92±0.82*	5.6±0.70*	62.7*
4	Test-2	EAFPG(200mg/kg)	2.50±0.50**	1.6±1.0**	78.7**

EEPG- Ethanol extract of *Psidium guajava*; EAFPG - Ethyl acetate fraction of *Psidium guajava;* Results were expressed as mean ± SEM, Statistical test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6); Values are significant **P<0.01 when compared against control; Values are significant **P<0.01,*p<0.05 when compared against control.

CONCLUSION

The EEPG and EAFPG showed a dose dependent inhibition of nitric oxide free radicals. Reactive nitrogen species (RNS) has been suggested to be involved in gastric mucosal damage. The RNS scavenging activity of the EEPG and EAFPG may be contributed to the presence of flavonoids (quercetin). As flavonoids have been identified in the ethanolic extract and its fraction, we believe that the antiulcer activity of this extract is probably due to the antioxidant activity of the extract. Antioxidant activities of flavonoids have been well documented in the literature. Moreover, flavonoids have been reported earlier for their antiulcerogenic activity and gastric protection. So the present study. Maximum inhibitory effect of the ethyl acetate fraction of *Psidium guajava* Linn due to least partly to the presence of flavonoid.From this study, it is concluded that *Psidium guajava* Linn. Leaf extract have significant anti-ulcer activity in animal models. Compared to ethanolic extract ethyl acetate fraction showed significant antiulcer protection at tested concentration. It also has mucoprotective activity and gastric antisecretory when compared with that of reference drug Ranitidine. The anti-ulcer activity is probably due to the presence of flavonoids (quercetin).

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