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Research Article

Phytochemical profile and anti-ulcer activities of extracts of unripen fruits of *psidium guajava linn* by ethanol induced ulcer method

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Abstract
Psidium guajava L. is a small medicinal tree that is native to South America. It is popularly known as guava (family Myrtaceae) and has been used traditionally as a medicinal plant throughout the world for a number of ailments. The ulcer that is exposed to pepsin is referred to as peptic ulcers. Peptic ulcers are found in the lining of our stomach. Pepsin is normally present within the hydrochloric acid inside the stomach lining. The phyto active constituents like flavonoids, tannins and terpenoids, have been reported in several anti-ulcer literatures as possible gastro protective agents. Flavonoids, tannins and triterpenes are among the cytoprotective active materials for which anti ulcerogenic efficacy has been extensively confirmed. It was suggested that these compounds have been able to the stimulate mucus, bicarbonate and prostaglandin secretion, and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen. These phyto constituents present in the extract could be the possible agents involved in the prevention of gastric lesions induced by ethanol-induced gastric ulcer. *Psidium Guajava Linn* showed a dose dependent curative ratio compared to ulcer control groups. The methanol extracts exhibited an inhibition percentage of 55.60% and PEPG extracts 42% and AQPG extracts 50.27% at doses of 400mg/kg, 400mg/kg and 500mg/kg doses respectively. The ulcer protective action of extracts at 400mg/kg was good to that of standard drug, Ranitidine, which exhibited an inhibition percentage of 72.97%.

Keywords: anti-ulcer activities, *psidium guajava linn*, induced ulcer, ulcer Index, Pharmacological studies.

INTRODUCTION

Medicinal plants have been identified and used through human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects. The use of plants as medicines predates written human history. Ethno botany is recognized as an effective way to discover future medicines.

The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. Conservation of natural resources and the capability to utilize them in sustained manner are essential for the well being and continued survival of man. Under the duress of over exploration and habitat degradation a number of wild plants are essentially facing a constant threat of extinction. Many of these plants are rare and endemic and found only in wild sources. The population explosion coupled with the improved

standard of living led to ruthless exploitation, resulting in the imminent danger of extinction of these plants[1]. Most of these wild medicinal and aromatic plants are highly habitual specific, found only in forests and occupying highly specialized ecological niche with restricted distribution. This system was developed in the Western countries. In this system drugs are manufactured using synthetic chemicals and / or chemicals derived from natural products like plants, animals, minerals etc. Different countries of the world developed independently their own traditional systems of medicine using locally available materials like minerals and products of plants and animals[2]. The World Health Organisation is giving considerable importance to these alternate medicine systems to provide Primary HealthCare to millions of people in the developing countries.

Psidium guajava L. is a small medicinal tree that is native to South America. It is popularly known as guava (family Myrtaceae) and has been used traditionally as a medicinal plant throughout the world for a number of ailments. There are two most common varieties of guava: the red (*P. guajava* var. *pomifera*) and the white (*P. guajava* var. *pyrifera*). Open sore on an external or internal surface of the body, caused by a break in the skin or mucous membrane which fails to heal. Ulcers range from small, painful sores in the mouth to bedsores and serious lesions of the stomach or intestine [3]. The H_2 blockers interfere with acid production by blocking histamine, a substance produced by the body that encourages acid secretion in the stomach. H_2 blockers were the standard treatment for peptic ulcers until proton pump inhibitors and antibiotic regimens against H. *pylori* were developed. These drugs cannot cure ulcers, but they are useful in certain cases. All have good safety profiles and few side effects. There are some differences between these drugs [4].

Chemical composition

The fruit: The fruits also contain vitamin C and vitamin A, iron, calcium and phosphorus. Guavas are up to 5 times richer in vitamin C than oranges. Manganese is also present in the plant in combination with phosphoric, oxalic and malic acids. The fruit contains saponin combined with oleanolic acid. Morin-3-O-α-L-lyxopyranoside and morin-3-O-α-L-arabopyranoside and flavonoids, guaijavarin and quercetin

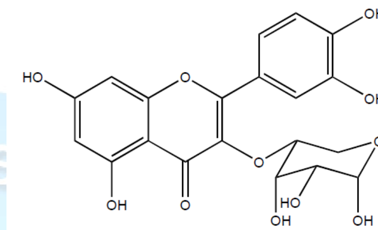

Figure: 1 Structure of guaijavarin

Collection of plant material[5]: The unripen fruit of *Psidiumguajava* was collected from venkatagiri hills of Nellore district of Andhra Pradesh of India in the month of December 2015. The un ripen fruit were separated, cleaned, air dried and grinded into powder. The powdered material was passed through sieve and stored in airtight container.

Extraction: The dried powder (250 gms) of *Psidiumguajava* was extracted with petroleum ether (40-60 C), methanol. The powder was extracted by for a Soxhlet apparatus. It was run up with 72 hours for each solvent. Then each extract was collected and subjected to distillation to recover the mthanol, petroleum ether [6]. Water extracted by the method of maceration. Now the extract was further concentrated and was dried in desicators.

Phytochemical investigation: The Preparation of alcoholic extract: The 250 gm of air dried and coarsely powdered material un ripen fruit of *Psidiumguajava* was extracted withmethanol,pet.etherby hot percolation method (hot extraction) for 72 hrs.water is from maceration Then the extract was filtered with meslin cloth and then evaporated to reduce pressure and vaccum dried. The yield is respectively each solvents yellow residue of methanol 35% w/w, pet ether 28% w/w, and water 25% w/w.

Phytochemical investigation: The plants may be considered as biosynthetic laboratory and contains multiple compounds like alkaloids, glycosides, volatile oils and tannins etc that exerts physiological effects. The compounds that are responsible for therapeutic effects are usually secondary metabolites [7]. Aqueous and alcoholic extracts and different

fractions of plant material were subjected to preliminary phytochemical screening for detection of various plant constituents.

Test for sterols[8]:

Salkowski reaction: Few mg of the residue of each extract was taken in 2ml of chloroform and 2ml of conc. sulphuric acid was added from the side of the test tube. The test tube was taken was shaken for few minutes. The development of red colour in the chloroform layer indicated the presence of sterols.

Liberman's test: To a few mg of the residue in a test tube few ml of acetic anhydride was added and gently heated. The contents of the test tube were cooled. Few drops of conc. sulphuric acid were added from the side of the test tube. A blue colour gave the evidence of presence of sterols.

Libermann-buchard's reaction: Few mg of residue was dissolved in chloroform and few drops of acetic anhydride were added to it, followed by conc. sulphuric acid from the sides of the tube. A transient colour development from red to blue and finally green indicated the presence of sterols.

Test for alkaloids[9]:

Dragendroff's test: It was prepared by mixing two solutions. Solution A (17mg of bismuth subnitrate + 200gm tartaric acid + 800ml distilled water) and Solution B (160gm potassium iodide + 4ml distilled water) were mixes in 1:1 v/v proportion. From this solution working standard was prepared by taking 50ml of this solution and adding 100gm of tartaric acid and making upto 500ml with distilled water. The above dragendroff's reagent was sprayed on whatmann No.1 filter paper and the paper was dried. The test filtrate after basification with dilute ammonia was extracted with chloroform and the chloroform extract was applied on the filter paper, impregnated with dragendroff's reagent with the help of a capillary tube. Development of an orange red colour on the paper indicated the presence of alkaloids.

Mayer's test: The Mayer's reagent was prepared as follows: 1.36gm of mercuric chloride was dissolved in 60ml of distilled water. Both the solutions were mixed and diluted to 100ml with distilled water. To a little of the filtrate, taken in a watch glass, a few drops of the above reagent were added. Formation of cream coloured precipitate showed the presence of alkaloids.

Wagner's test: 1.27gm of iodine and 2gm of potassium iodide were dissolved in 5ml of water and solution was diluted to 100ml with water. When few drops of this reagent were added to the test. Filtrate, a brown flocculent precipitate was formed indicating the presence of alkaloids.

Hager's test: A saturated aqueous solution of picric acid was employed for this test. When the test filtrate was treated with this reagent, an orange yellow precipitate was obtained, indicating the presence of alkaloids.

Test for saponins[10]: A few mg of the test residue was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water if a stable characteristic honey comb like forth is obtained, saponins are present.

Heamolysis test: A little amount of test residue was dissolved in normal saline in such a way that 5ml of the solution represent1 gm of the crude drug. In a series of 5 test tubes, doses of 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1ml was added and volume was made up to 1ml in each case with normal saline. 1ml of diluted blood (0.5ml of rabbit's blood diluted to 25ml with normal saline) was added to each tube and changes observed. If heamolysis of blood occurs, then saponins are present.

Test for tannins: The test residue of each extract was taken separately in water, warm it and filtered. Test was carried out with the filtrate using following reagents.

Ferric chloride test: A 5% w/v solution of ferric chloride in 90% alcohol was prepared and few drops of this solution were added to a little of the above filtrate. If dark green or deep blue colour is obtained, tannins are present.

Lead acetate test: A 10% w/v solution of basic lead acetate, distilled water was added to the test filtrate. If precipitate is obtained, tannins are present.
Potassium dichromate test: On addition of solu-

tion of potassium dichromate in a test filtrate, dark colour is developed, tannins are present.

Gelatin solution test: 1%w/v solution of gelatin in water, containing 10% sodium chloride was prepared, alittle of this solution was added to the filtrate. If white precipitate is obtained then tannins are present.

Bromine water test: Bromine water was added to the test filtrate. If decolourisation of bromine water occurs then tannins are present.

Test for flavonoids[11]:

Shinoid test: A small quantity of test residue was dissolved in 5ml ethanol (95% v/v) and reacted with few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. The pink, crimson or magenta colour is developed within 1 or 2 mins then flavonoids are present.

Test for proteins[12]:

Biuret test: A few mg of residue was taken to it add water and 1ml of 4% sodium hydroxide solution.Violet or pink colour is formed then proteins are present.

Xanthoproteic test: A little residue was taken add 2ml of water and 0.5 ml of concentrated nitric acid.Yellow colour is obtained if proteins are present.

Million's test: Million's reagent (mercuric nitrate solution)was prepared by dissolving 3ml of mercury in 27ml of fuming nitric acid, keep the mixture aside until it cooled, this solution was then diluted with equal quantity of distilled water. The Aqueous solution of the residue was taken and to it, 2 to 3ml of million's reagent was added. The white precipitate which slowly turns into pink, is obtained if proteins are present.

Test for amino acid [13]:

Ninhydrin test: 0.1% w/v solution of ninhydrin was added in an n-butanol. A little of this reagent was added to a test extract. A violet or purple colour is developed if amino acids are present.

Test for sugars:

Molisch's test: The molisch's reagent was prepared by dissolving 10g of a naphthol in 100ml of 95% alcohol. A few mg of the test residue was placed in a test tube containing 0.5ml of water and it was mixed with 2drops of molisch's reagent. To this solution 1ml of concentrated sulphuric acid from the side of inclined test test tube was added, so that acid forms a layer beneath the aqueous solution. If red brown ring appears at the common surface then liquid sugars are present.

Barfoed's test: This reagent was prepared by dissolving 13.3 gm of crystalline neutral copper acetate in 200ml of 1% acetic acid solution. The test residue was dissolved in water and heated with little of reagent. If a red precipitate of cuprous oxide is formed with in 2 min then monosaccharides are present.

Fehling's test: The fehling's solution was prepared as follows:

Solution A: Copper sulphate -34.64g, Sulphuric acid – 0.5 ml, Distilled water to 500ml

Solution B: Sodium potassium tartarate -176g, Sodium hydroxide 77g, Distilled water to 500ml

The two solutions were mixed in equal volumes immediately before use. A little, of the test residue was dissolved in water and a few ml of fehling's solution was added to it. This mixture was then warmed. If a red precipitate of cuprous oxide is obtained, then reducing sugars are present.

Pharmacological studies

Acute oral toxicity study: The protocol used in this study was submitted to the institutional animal ethics committee. Acute oral toxicity study was performed according to the guidelines acute toxic class method described by OECD. Swiss albino mice of female sex (as they are more sensitive) weighing 20-25 gms were used for the study. The animals were fed with standard mouse diet, had free access to water and maintained in a well ventilated condition of 12 hour light cycle [14]. They were kept in clean polypropylene cages with wood shavings as bedding and were adapted to laboratory condition for 7 days prior to the experiment. The animals were fasted prior to dosing feed, but not water was with held for 3-4 hours. The fasted animals were weighed to determine the appropriate quantity of extract (mg kg-1) to be administered. In all cases, the maximum volume of aliquot portion of the extract used did not exceed

1ml/100mg of the animal body weight as per guidelines. The animals were randomly assigned to cages for grouping and individual animals were for marked. Acute toxic class method is stepwise procedure with use of 3 animals of a single sex per step. Depending on the mortality or 67the morbidity, status of the animals average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. Three animals were used for each step[15]. The starting dose of extract 5 mg/kg body weight suspended in 1% w/w acacia was administered orally using intra gastric tubes. The dosing for 2 second animal is delayed to observe the toxicity of the previously dosed animal. Once no toxicity signs were observed in previously dosed animal. The second and third animals were dosed.

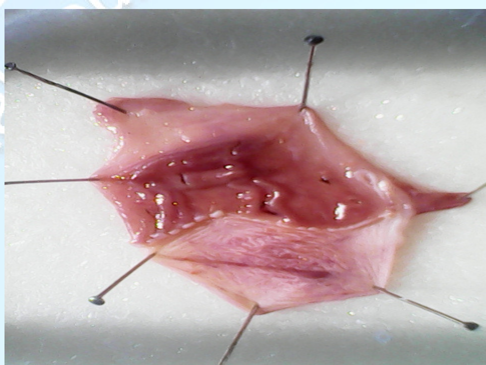

Figure: 2 Standard drug (Ranitidine)

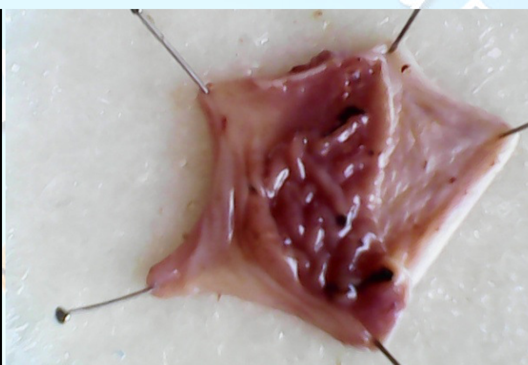

Figure: 3 Control (Ethanol)

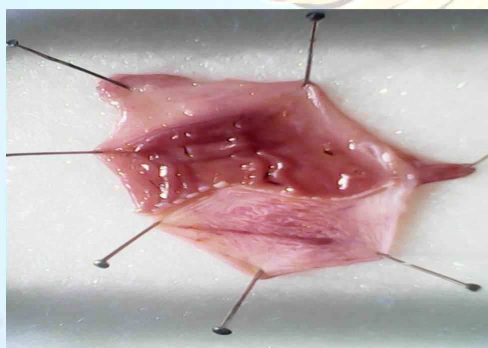

Figure: 4 MEPG Extract

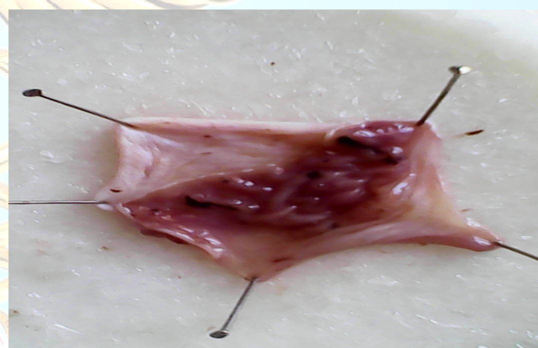

Figure: 5 AQPG Extract

Figure: 6 PEPG Extract

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Table No: 7 Preliminary Phyto chemical tests of extracts

Phytoconstituents	Extracts		
	Petroleum ether	Methanol	Water
Carbohydrates	-	+	+
Glycosides	-	+	-
Alkaloids	-	+	-
Phytosteroids	+	+	+
Flavonoids	+	+	+
Protein and amino acids	-	-	-
Tannins	-	+	-
Saponins	-	+	+
Gum and mucilage	-	+	-
Terpenoids	-	+	-
Resins	-	-	-

Table: 8 Effect of antiulcer activity of parameters in different extracts

Sl no	Compound	Dose	Ph	Total acidify (mEq/L)	Ulcer index	% inhibition of ulcer index
1	Control	5ml/200g	2.81±0.03	102.2 ± 0.06	3.70	
2	MEPG	400mg/kg	3.85 ± 0.03	60 ± 0.24	1.64	55.6
3	Ranitidine	20/kg	5.35 ± 0.22	30.4 ± 0.48	1	72.97
4	PEPG	400/kg	3.23±0.05	55.4±0.26	2.12	42.70
5	AQPG	500/kg	3.15±0.03	50.8±0.22	1.84	50.27

The animals were placed individually and were observed for any sign of acute toxicity, morbidity or mortality during the first 24 hours, with special attention given during the first 4 hours, with special attention given during the first 4 hours and daily thereafter for a total of 14 days second step of acute oral toxicity is performed with 50 mg/kg body weight suspended in 1% w/v acacia. The animals were placed individually and were observed for any sign of acute toxicity, morbidity or morality during first 24 hours, with special attention given during first 4 hours and daily thereafter for a total of 14 days. The animals in third step were tested with 300mg/kg of ethanol extract followed by 2000mg/kg body wt of extract in fourth step. The animals were observed for toxic effects [16]. As per guidelines only when justified by specific regulatory needs, the use of additional upper dose level of 5000mg/ kg body weight may be considered. For reasons of animal welfare concern, testing in animals in ranges of 2000-5000mg/kg is discouraged.

Experimental procedure: Wistar Albino rats of either sex were divided into six groups, each group consists of six animals. All groups of animals received following treatments for 5 days: groups 1 (Normal) and 2 (Control) received vehicle 10 ml/kg, groups 3,4 and 5 were given respectively PEPG (400mg/kg) MEPG (400mg/kg), AQPG (500mg/kg), and the group 6 (Standard) given reference drug Ranitidine at the dose of 20 mg/kg orally once daily for 15days. On the 5th day, 1h after final dose of treatment, the gastric ulcers were induced in rats by administering 80% ethanol (5ml/200g) for ethanol-induced ulcer. After 1h, animals were sacrificed by cervical dislocation and stomach was incised along the greater curvature and examined for ulcers index[17]. Percentage ulcer inhibition was calculated for each group on comparison with vehicle control group.

RESULTS

Preliminary phytochemical tests: The Active constituents present in the plant material are of vital importance, as they are known to produce one or other definite pharmacological action. Phyto chemical test performed using standard procedure with specific reagents, result of phyto chemical investigation indicate the presence of carbohydrates, Glycosides, steroids, saponines, tannins and phenol compounds, Phytosteroids, Flavonoids.

Evaluation of Antiulcer activity in Ethanol induced gastric ulcer: In ethanol induced model ulcer, index has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. This is shown in Table no 8, Percentage inhibition of ulcer for MEPG (400mg/kg) and ranitidine (20mg/kg) significantly reduced the ulcer index, the total ,free acidity shown (table 2).The percentages of inhibition of ulcers were 55.60 % for the test group with 400mg/kg of MEPG and compared with standard group.

Ulcer scoring: Observations on stomach Ulcer score: Normal colored stomach 0.0, Red coloration 0.5, Spot ulcers 1.0, and Hemorrhagic streaks 1.5, Ulcers ≥ 3 ≤ 5 mm 2.0, Ulcers > 5 mm 3.0.

Summary and conclusion: The present study was undertaken to determine the antiulcer activity of the petroleum ether, methanol and water extract from the Unripe Fruits of *Psidium Guajava Linn*. The preliminary phytochemical investigation showed the presence of alkaloids, flavonoids, cardiac glycosides, and phytosteroids. Tannins, terpenoids. The pharmacological and acute toxicity studies of ethanol extract was performed by following, OECD-423 guidelines (Acute toxic class method). No mortality or acute toxicity was observed (3 days) up to 2000mg/kg of body weight. The phyto constituents like flavonoids, tannins and terpenoids, have been reported in several anti-ulcer literatures as possible gastro protective agents. Flavonoids, tannins and triterpenes are among the cytoprotective active materials for which anti ulcerogenic efficacy has been extensively confirmed. It is suggested that these compounds will be able to stimulate mucus, bicarbonate and prostaglandin secretion, and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen. Tannins may prevent ulcer development due to their protein precipitating and vasoconstriction effects. Their astringent action can help precipitating micro proteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants. Similarly, the methanol extract of *Psidium Guajava Linn* showed the presence flavonoids and their glycosides, tannins and triterpenoids. These phyto constituents present in the extract could be the possible agents involved in the prevention of gastric lesions induced by ethanol-induced gastric ulcer. *Psidium Guajava Linn* showed a dose dependent curative ratio compared to ulcer control groups. The methanol extracts exhibited an inhibition percentage of 55.60%, PEPG extracts 42%, and AQPG extracts 50.27% at doses of 400mg/kg, 400mg/kg and 500mg/kg doses respectively. The ulcer protective action of extracts at 400mg/kg was good to that of standard drug, Ranitidine, which exhibited an inhibition percentage of 72.97%

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