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RESEARCH ARTICLE

Phytochemical and Pharmacological Investigation on Extracts Prepared from Various Parts of *Psidium guajava*

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ABSTRACT

The objective of the study was to perform phytochemical screening of alcoholic and aqueous extracts of various parts of *Psidium guajava* and perform pharmacological screening for anti diabetic activity using alloxan induced diabetic rats and anticonvulsant activity by using PTZ induced seizure model. The phytochemical investigation showed the presence of glycosides, flavanoids, alkaloids, saponins, vitamin, carbohydrate, amino acid in the extracts. The aqueous leaf extracts showed significant anticonvulsant activity and more reliable antidiabetic activity compared to other extracts.

KEYWORDS

Psidium Guajava, Phytochemical Screening, Anti convulsant, PTZ, Anti Diabetic, Alloxan

INTRODUCTION

Diabetes was one of the major killers of mankind before the discovery of pancreatic extract insulin in1921 and oral hypoglycemic. It is one of the world's largest health problems with an estimate of minimum 40 million people suffering from this disorder. In this disease blood glucose level rises above 80-120 mg/dl. Oral antidiabetic sulfonyl ureas and biguanides are used by 30% of all diabetic patients but these are having side effects. In resent year the emphasis has been to identify as many plants as possible which could have effective control of the disease b if not able to completely cure the same. Pharmacological screening and clinical trial reveal the presence of hypoglycemic activity in large number of plants.

Epilepsy is a collective term for a group of chronic seizure disorder having in common, sudden and transient episodes (seizure) of loss or disturbance of consciousness, usually but not always with a characteristic body movements (convulsions) and sometimes with autonomic hyperactivity.[1,2] There are a number of synthetic anticonvulsant drugs currently available for use in the management, control and treatment of individuals with epilepsy. However, most of the synthetic drugs are not only inaccessible and unaffordable, but also possess many toxic adverse effects. Therefore, there is a great need for the development of cheap, effective and safe anticonvulsant agents from plants and other sources. In traditional systems of Ayurvedic medicine, *Psidium guajava* is a well known plant drug used for its Anti-Epileptic property[3].

Medicinal plants are used in different countries as sources of many potent and powerful medicines. *Psidium guajava* is a small evergreen sub deciduous tree[4]. It is found in tropical and subtropical areas[5]. It is a native of tropical

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America[6]. Guava fruit is still enjoyed as a sweet treat by indigenous peoples throughout the rainforest and the leaves and bark of the guava tree has a long history of medicinal uses that are still employed today. The leaves and barks of guava is used as a cure of diarrhoea[7], sore throat, vomiting, stomach upset, vertigo etc. In a recent study with guinea pigs Brazilian researches reported that guava leaf extract have numerous effect on cardiovascular system[8]. Other animal studies showed that guava leaf extracts have analgesic, sedative and CNS depressant activity. The taxonomical classification of the plant *Psidium guajava* is as follows

Kingdom :- Plantae

Order :- Myrtales

Family :- Myrtaceae

Subfamily :- myrtoideae

Genus :- Psidium

Species :- guajava

Literature reviews showed that guava is rich in tannin, phenols, flavanoids, saponins, carotenoids, vitamins, fiber and fatty acids. Guava fruit is rich in vitamin-C. Aim of the present study is to prepare aqueous and alcoholic extracts of tender leaves, fruit pulp and seeds of *Psidium guajava* and to compare antidiabetic and anticonvulsant activities of these extracts.

MATERIAL AND METHODS

Method for Collection of Plant

The tender leaves and fruit of the plant *Psidium guajava* were collected in the month of November 2009 from Agriculture Research Institute Durgapura, Jaipur, (Rajasthan). Authentication of the plant was done in the Department of Botany, Rajasthan University, Jaipur (Rajasthan) under registration no. 20608.

Preparation of Sample

The tender leaves were dried in shade. The fruit of *Psidium guajava* without peel were boiled with water with gentle heating to avoid decomposition of the constituents. This procedure was carried out to separate seed from fruit pulp. After which seed and fruit pulp were dried in shade and were crushed to obtain a coarse powder.

Preparation of Extracts

The dried plant materials were crushed into powder and were subjected to aqueous and alcoholic extraction using soxhlet extraction method. First the sample material was extracted with petroleum ether after which the samples were extracted with distilled water and 99.9% alcohol for a period of 24hrs until it becomes colourless. After extraction solvent was evaporated to dryness and percentage yield was calculated. This process was carried out separately for each selected plant part.

Phytochemical Analysis

Freshly prepared extracts of tender leaf, fruit pulp, and seeds of *Psidium guajava* were qualitatively tested for presence of various phytoconstituents.[9]

Experimental Animals

Healthy adult albino wister rats of either sex, 150-250 gm of body weight were selected for studies issued from JNU campus, Jaipur. They were kept in standard polypropylene cage at room temperature of 27^0C and well ventilated. All animals were maintained under standard housing conditions. They were fed a standard rat pellet and water. Animals used for the study were approved by institutional animal ethical committee (IAEC) (Animal Institutional no. 1054/ac/07/CPCSEA). The care of laboratory animal was taken as per the CPCSEA regulation. (REG NO. 018/2010/CPCSEA/JNU).

Acute Toxicity Study[10]

Acute oral toxicity test was done in aqueous extract of tender leaves, fruit pulps and seeds of *Psidium guajava* as per OECD guidelines 420. A limit of dose was selected for every extract. It was upto 2000mg//kg. A total of 15 animals were tested. (5 in each group). Animals were fasted for 3-4hrs before and 1-2 hrs after the experiment. Animals were observed for 14 days for mortality and behavioral changes.

Pharmacological Screening

Antidiabetic Activity in Alloxan Induced Diabetic Rats[11]

Diabetes was induced by single ip injection of freshly prepared aqueous solution of alloxan monohydrate (150mg/kg) to overnight fasted rat. After 48hr of alloxan injection the animal which did not develop hyperglycemia ie, glucose level >200mg/dl were rejected and replaced with new animals immediately. For this male wister albino rats of 150-250 kg body weight was selected. After confirmation of diabetes animals were divided into six groups (six in each)first group served as control (Normal saline), served as diabetic control (alloxann induced), third group received aqueous seed extract (200mg/kg), fourth group received aqueous leaf extract(200mg/kg), fifth group received aqueous fruit pulp extract (200mg/kg), sixth group served as standard group (glibenclamide 2.5mg/kg) treatment was continued for consecutive 7 days with once a day dose (Morning). Before the treatment (0 day) and at 3, 5, and 7 day blood was collected from tip of the tail and glucose level was checked by glucometer.

Anticonvulsant Activity[12]

Pentylenetetrazole (PTZ) induced seizure model was used to determine anticonvulsant activity of aqueous extracts of *Psidium guajava.* Rats were divided into 5 groups each containing 6 animals. First group served as control (distilled water), second group served as standard group(diazepam 4mg/kgip), third group received aqueous seed extract (200mg/kg), fourth group received aqueous leaf extract (200mg/kg),fifth group received aqueous fruit pulp extract (200mg/kg). After a pretreatment time of 60 minutes PTZ (85mg/kg ip) was administered to all groups of animals. After it the onset of convulsion and duration of convulsion were recorded.

Statistical Analysis

All data were represented as mean ±SEM values. Data were analyzed by one-way ANOVA. Whenever ANOVA was significant, further comparison was made against the vehicle treated groups were performed using the Dunnett's - tests. The level of statistical significance adopted was $P<0.001$.

RESULTS AND DISCUSSION

Aqueous and alcoholic extracts of tender leaves, fruit pulp, and seeds of Psidium guajava were obtained by soxhlet extraction. Alcoholic extracts were obtained in more proportion compared to aqueous extracts. Phytochemical screening of both aqueous and alcoholic extract showed the presence of glycosides, flavanoids, alkaloids, saponins, vitamin, carbohydrate, amino acid. The result of phytochemical screening is shown in Table 1. Leaf extract showed the presence of maximum number of phytoconstituents was an alcoholic fruit pulp extract showed minimum constituents.

Pharmacological Screening

Pharmacological screening of aqueous extract of tender leaves, fruit pulp and seed was performed for antidiabetic and anticonvulsant activities.

Acute Toxicity Study

Acute toxicity study was performed and the result showed that no death occurred up to 2000mg/kg. 1/10 of safe dose ie, 200mg/kg was selected for administering in the animals.

Antidiabetic Activity in Alloxan Induced Diabetic Rat Models

Antidiabetic activity was tested in alloxan induced diabetic rat models. Aqueous leaf, fruit pulp, and seed extract showed significant antidiabetic activity compared to diabetic control. The percentage inhibition of blood glucose level is more (72.11%) by aqueous leaf extract than compared to fruit pulp (70.3%) and aqueous seed

Table 1: Results of phytochemical screening

Sl. No.	Phyto-constituent	Alcoholic leaf extract	Aqueous leaf extract	Alcoholic Seed extract	Aqueous Seed extract	Alcoholic Fruit pulp extract	Aqueous Fruit pulp Extract
1	Carbohydrate	+	+	+	+	+	+
2	Flavanoids	+	+	-	-	+	+
3	Alkaloids	+	+	-	+	-	+
4	Glycosides	+	+	+	+	+	+
5	Saponin	-	+	-	+	-	+
6	Tannin	+	+	-	-	-	-
7	Vitamin	+	+	+	+	+	+
8	Amino acids	-	-	-	+	-	+
9	Steroids	+	+	+	-	-	-

(+) Presence of phytochemical constituent

(-) Absence of phytochemical constituent

Table 2: Antidiabetic activity in alloxan induced diabetic rat models

Group	Treatment dose(mg/kg)	Blood Glucose Level			
		1 day	2 day	3 day	4 day
Normal	Normal saline	74.3±1.85	75.5±1.59	77.4±2.5	78.3±2.3
Diabetic control	Alloxan induced(150mg/kg)	260.3±1.46	261±1.50	263±1.52	268±1.71
Test I	Aqueous leaf extract (200mg/kg)	234.6±2.10	194.43**±2.4	144.6**±1.40	131.2**±2.7
Test II	Aqueous fruit pulp extract (200mg/kg)	247.6±1.90	162.5**±1.5	140.3**±3.1	134.7**±3.8
Test III	Aqueous seed extract (200mg/kg)	250±1.7	190.4**±3.8	170.2**±2.2	160.1**±2.1
Standard	Glibenclamide	204.7±2.7	125.7**±1.10	118.4**±4.3	102.8**±3.2

Values are mean in SEM, n=6, **=P<0.01 are significant as compared to diabetic control of respective group (one way ANOVA followed by Dunnett's 't' test)

extract (56.85%). More antidiabetic activity of aqueous leaf extract at a dose of 200mg/kg may be due to the presence of phytoconstituents such as flavonoid, glycoside, alkaloid etc.

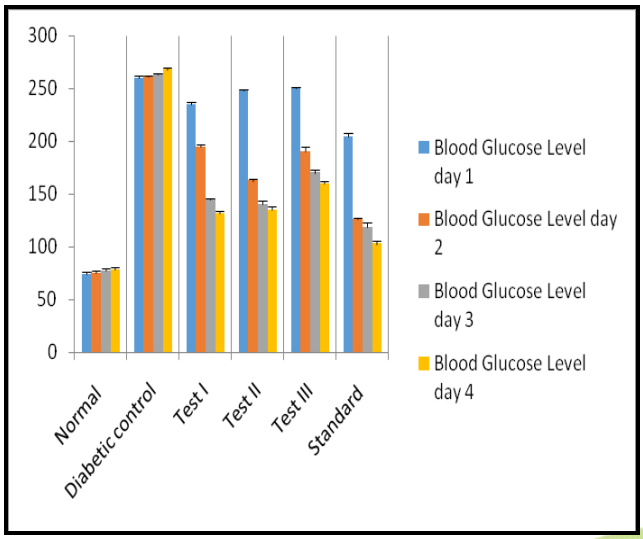

Figure 1: antidiabetic activity in alloxan induced diabetic rat models

Anticonvulsant Activity

Anticonvulsant activity done in PTZ induced seizure models, administration of aqueous leaf extracts of Psidium guajava at a dose of 200mg/kg one hour prior to the injection of PTZ (as compared to other extracts) significantly (P<0.05) delayed the onset of convulsions.

Table 4: anticonvulsant activity of aqueous seed, fruit pulp, and tender leaf extracts in PTZ induced seizure models

Sl No.	Treatment	Onset of action	Duration of action
1	Control	1.21±4.62	18.456±3.650
2	Standard (diazepam)	0.0±0.0	0.0±0.0
3	Aqueous seed extract (200mg/kg)	1.41±4.62	13.054±4.765
4	Aqueous leaf extract (200mg/kg)	3.45±4.62	17.548±3.065
5	Aqueous fruit pulp extract (200mg/kg)	2.76±0.8760	14.56±3.089

Values are expressed in mean ± SEM, n=6,* = p<0.05 compared with respective control (one way ANOVA followed by Dunnett's't' test)

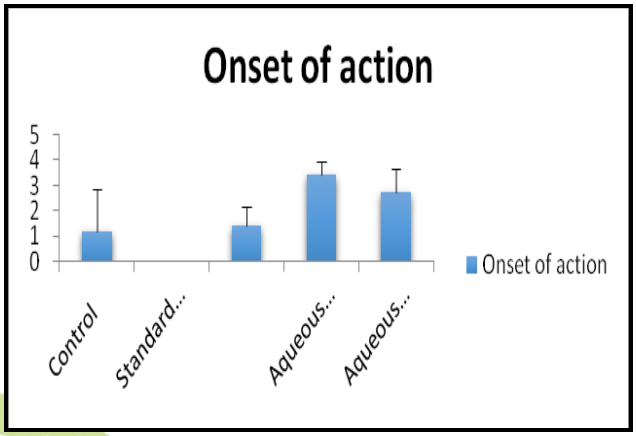

Figure 2: Anticonvulsant activity

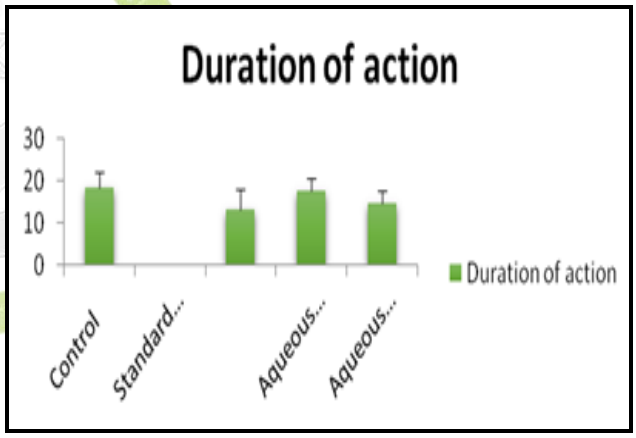

Figure 3: Anticonvulsant activity

CONCLUSION

The objective of the study was to perform phytochemical screening of alcoholic and aqueous extracts of various parts of *Psidium guajava* and perform pharmacological screening for anti diabetic activity using alloxan induced diabetic rats and anticonvulsant activity by using PTZ induced seizure model. Tender leaves, fruit pulp and seeds were used for extraction process. Aqueous and alcoholic extracts were prepared by soxhlet extraction method.

The phytochemical investigation showed the presence of glycosides, flavanoids, alkaloids,

saponins, vitamin, carbohydrate, amino acid in the extracts. The aqueous leaf extracts showed significant anticonvulsant activity in PTZ induced seizures at a dose of 200mg/kg.

All aqueous extracts showed significant antidiabetic activity when compared to diabetic control. Maximum antidiabetic activity was obtained for aqueous leaf extracts compared to other extracts.

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