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# Phytochemical and pharmacological evaluation of seeds of *Psidium guajava* Linn.

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**ABSTRACT**

*Though the seeds of Psidium guajava is edible and consumed along with the edible part of the fruit, it was not evaluated for its pharmacological activities fully. Hence the seeds of the guava fruit are evaluated for Phytochemical and pharmacological evaluation and found many constituents like, fixed oil, sugars, anthocyanins, amino acids and remarkable anti diabetic activity for its alcohol and ethyl acetate extracts. These activities are dose dependent and at higher doses Ethyl acetate extract has even better activity than the standard.*

**Key words**: Psidium guajava, Seed, Ethyl acetate extract, anti diabetic.

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## INTRODUCTION

It was decided to study the seeds of Guava fruit, as it is edible unlike seeds of other fruits like grapes. As it is consumed in considerable quantity along with the fruit, it was taken for the present work to evaluate its beneficial effects. It was planned to separate the seeds from the pulp of the fruit and its constituents and their pharmacological activities are aimed to be evaluated .Folklore literature ascribe many activities for this fruit including anti diabetic effect. Hence it is studied in this project after extracting the constituents with various solvents. As almost all seeds have fixed oil, it is to be removed first and the defatted seed powder is to be extracted with hydro alcohol and ethyl acetate. Preliminary phyto chemical analysis and confirmation of phytoconstituents by chromatographic studies are planned and they are all presented in this paper. Finally the extracts are evaluated for anti diabetic activity by in vitro alpha amylase assay and reported.

## MATERIALS AND METHODS

Collection: The seeds of Psidium guajava.Linn was collected from the fresh fruits purchased from local market [‘More’ stores, Adyar, Chennai] and it was authenticated by the Taxonomist, D.B.Jain College of Arts and Science, Chennai. The seeds which are numerous, tiny and semi hard, concentrated at the centre of the fruit are removed carefully and washed repeatedly. They are dried under shadow without contamination by dust and insects. Then it was powdered in a mill without producing heat and sieved through No. 10 sieve to have uniform particles. It was packed in a well closed and well filled container, until further studies.

Physical evaluation: The powder was then subjected to physical evaluation. Its Extractive values, Ash values and Loss on drying were determined as per the methods given in Indian Pharmacopoeia and WHO manual ‘Quality

Control Manual for Medicinal Plant Materials'. These values are obtained for six samples and the average of each value is given in Table 1 below.

**TABLE: 1 PHYSICAL PARAMETERS**

| S.No. | PARAMETERS | AVERAGE OF 6 VALUES |
|---|---|---|
| 1. | Water Soluble Extractive | 5.2% w/w |
| 2. | Alcohol Soluble Extractive | 8.07% w/w |
| 3. | Loss on Drying | 2.2% w/w |
| 4. | Total Ash | 4.56% w/w |
| 5. | Acid Insoluble Ash | 0.014% w/w |
| 6. | Water Soluble Ash | 4.33% w/w |

Extraction: The seed powder was first extracted with Petroleum Ether [$40^o$-$60^o$ c] repeatedly using soxhlet apparatus. The oil extracted was recovered from petroleum ether extract by evaporation of the solvent. It was found to be pale yellow in colour and faint characteristic odour. The defatted material was then subjected to extraction with ethyl acetate and ethyl alcohol 80%, separately using soxhlet apparatus. Both the extracts were concentrated under vacuum and evaporated to dryness and then used for further phytochemical studies.

Phytochemical Evaluation: The oil, ethyl acetate extract and alcohol extracts were then subjected to preliminary phytochemical analysis using various chemical tests to identify the phytoconstituents present and the results are tabulated below in Table 2.

**TABLE .2 PRELIMINARY PHYTOCHEMICAL ANALYSIS**

| **PLANT CONSTITUENT**<br>**TEST/ REAGENTS** | **PETROLEUM ETHER EXTRACT** | **ETHYL ACETATE EXTRACT** | **ALCOHOL 80% EXTRACT** |
|---|---|---|---|
| 1. ALKALOIDS: | | | |
| A. MAYER'S REAGENT | -- | -- | -- |
| B. DRAGENDROFF'S REAGENT | -- | -- | -- |
| C.HAGER'S REAGENT | -- | -- | -- |
| D.WAGNER'S REAGENT | -- | -- | -- |
| 2. CARBOHYDRATE&GLYCOSIDES. | | | |
| A.MOLISH'S REAGENT | -- | + | + |
| B. FEHLING'S SOLUTION | -- | + | + |
| C. BENEDICT'S REAGENT | -- | + | + |
| D.LIBERMANN BURCHARD | -- | + | + |
| E. LEGAL'S TEST | -- | -- | -- |
| D. BORNTRAGER'S TEST | -- | -- | -- |
| 3. PHYTOSTEROLS: | | | |
| A.LIBERMANN'S TEST | -- | -- | -- |
| B.LIBERMANN BURCHARD'S TEST | -- | -- | -- |
| 4.FIXED OIL AND FATS: | | | |
| A.SPOT TEST | + | -- | -- |
| B. SAPONIFICATION TEST | + | -- | -- |
| 5.SAPONINS: | | | |
| A. FOAM TEST | -- | -- | -- |
| B. HAEMOLYSIS TEST | -- | -- | -- |
| 6. PHENOLIC COMPOUNDS AND TANNINS | | | |
| A.FERRIC CHLORIDE SOLUTION | -- | + | + |
| B.GELATIN SOLUTION | -- | + | + |
| C.LEAD ACETATE SOLUTION | -- | + | + |
| 7. PROTEINS AND AMINO ACIDS: | | | |
| A.MILLON'S REAGENT | -- | + | + |
| B.BIURET REAGENT | -- | + | + |
| C.NINHYDRIN TEST | -- | + | + |
| 8. GUMS AND MUCILAGES: | | | |
| A. ALCOHOL | -- | -- | -- |
| B. MOLISH TEST | -- | -- | -- |
| 9. VOLATILE OIL: | | | |
| A. HYDRO DISTILLATION | -- | -- | -- |

Chromatography: The preliminary phytochemical analysis indicated the presence of anthocyanins, amino acids and sugars. These constituents of seeds were confirmed by various chromatographic experiments which are given below:

**PAPER CHROMATOGRAPHY OF ANTHOCYANIDINS**

**Fig: 1**

Solvent: Forestal
Experiment 1: Rf value: 0.98

**Fig. 2**

Solvent: Forestal
Experiment 2: Rf value: 0.97

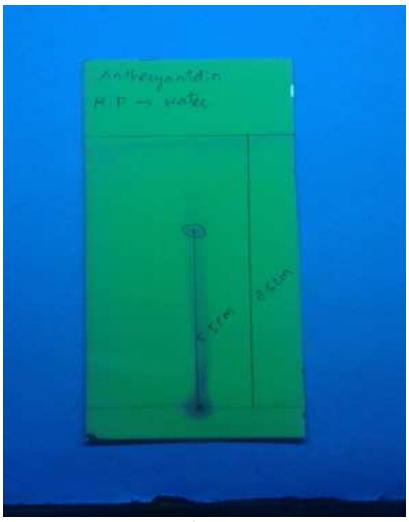


**Fig: 3**

Solvent: Water, Detection: Under U.V
Rf value: 0.65

**TABLE 3 CHROMATOGRAPHY OF SUGARS**

| Spots | Colour | Rf values | |
|---|---|---|---|
| | | Exp 1 | Exp 2 |
| Spot 1 | Brown | 0.57 | 0.62 |
| Spot 2 | Brown | 0.3 | 0.40 |
| Spot 3 | Brown | 0.11 | 0.06 |

*Solvent: B: A: W, 4:1:5*
*Detection Reagent: Aniline hydrogen phthalate*

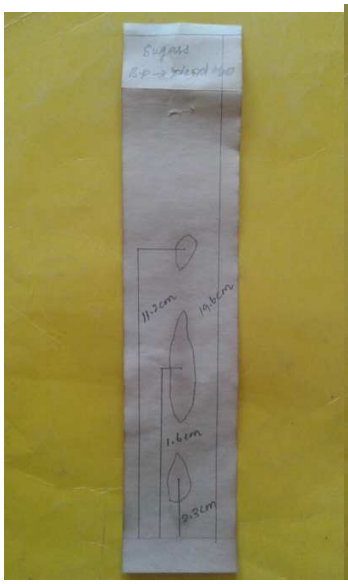


**Fig.4 [Exp. 1]**

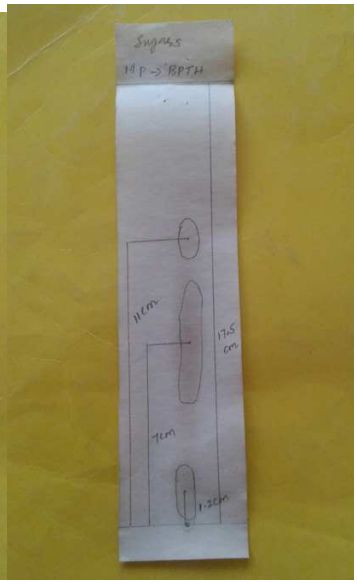


**Fig.5 [Exp.2]**

**Table 4 CHROMATOGRAPHY OF AMINO ACIDS**

| Spots | Colour | Rf value | | | |
|---|---|---|---|---|---|
| | | Exp 1 | Exp 2 | Exp 3 | Exp 4 |
| Spot 1 | Blue | 0.56 | 0.39 | 0.86 | 0.88 |
| Spot 2 | Blue | - | 0.09 | - | - |

Solvent: B: A: W [4:1:5]
Detection reagent: Ninhydrine

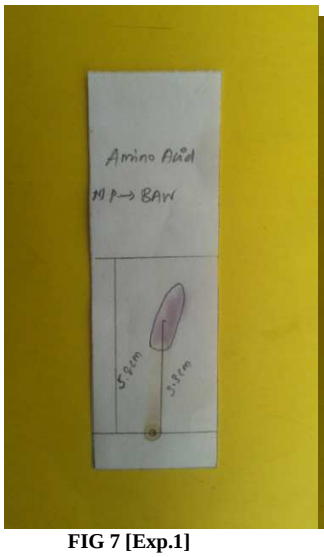


**FIG 7 [Exp.1]**

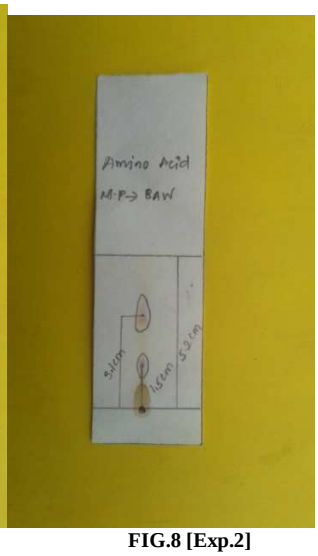
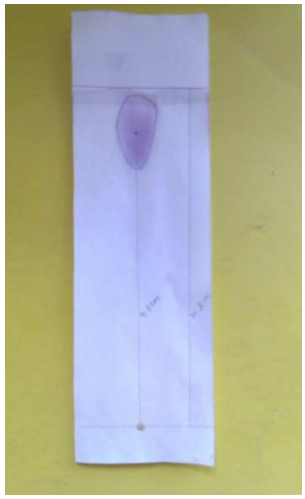


**FIG.8 [Exp.2]**



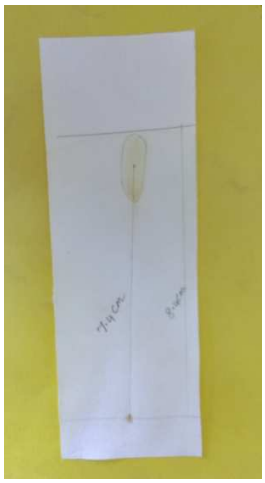


**FIG.9 [Exp.3]** **FIG. 10 [Exp.4]**
**[CHROMATOGRAPHY OF AMINO ACIDS]**

All the above experiments were carried out as per the methods given in Horborne which established the presence of one anthocyanidine, two amino acids and three sugars in the seeds of guava.

**Pharmacological Evaluation**: Anti diabetic potential of the alcohol 80% and Ethyl acetate extracts of the seeds of Guava were tested by in vitro alpha amylase method described in the Journal of Agriculture and Food Chemistry 2014; 62. 9507- 9514. Alpha amylase was dissolved in phosphate buffer saline (PBS, 0.02 mol/l, pH 6.8) at a concentration of 0.1 mg/ml. Various concentrations of sample solution (0.25 ml) were mixed with alpha amylase solution [0.25ml] and incubated at 37°C for 5 minutes. Then the reaction was initiated by adding 0.5 ml of 1.0% (w/v) starch substrate solution to the incubation medium. After incubation at 37°C for 3 minutes, the reaction was stopped by adding 0.5 ml of DNS reagent (1% Dinitro salicylic acid, 0.05% $Na_2So_3$ and 1% NaOH) to the reaction mixture and boiling at 100°C for 5 minutes. After cooling to room temperature the absorbance (Abs) at 540 nm was recorded by spectrophotometer. The inhibition percentage was calculated by the following equation.

% Inhibition = [(Abs 1-Abs 2)/Abs 1] × 100
where, Abs 1= Sample and Abs 2 = Control

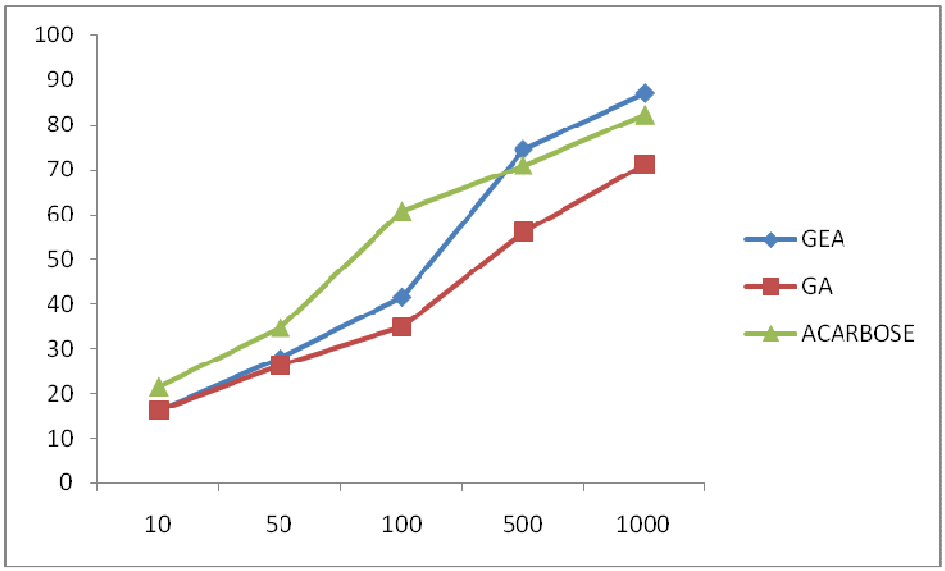
The results are tabulated below. They are also presented in the form of a graph.

**TABLE 5, ANTI DIABETIC ACTIVITY**

| Concentration | Absorbance | | | % Inhibition | | |
|---|---|---|---|---|---|---|
| | Sample 1 GEA | Sample 2 GA | Acarbose | Sample 1 GEA | Sample 2 GA | Acarbose |
| 10 µg | 0.074 | 0.074 | 0.079 | 16.22 | 16.22 | 21.52 |
| 50 µg | 0.086 | 0.084 | 0.095 | 27.91 | 26.19 | 34.73 |
| 100 µg | 0.106 | 0.095 | 0.158 | 41.52 | 34.73 | 60.75 |
| 500 µg | 0.243 | 0.141 | 0.213 | 74.48 | 56.02 | 70.89 |
| 1000 µg | 0.447 | 0.214 | 0.349 | 87.01 | 71.02 | 82.23 |
| Control | 0.062 | 0.062 | 0.062 | - | - | - |

*Sample 1: GEA: Guava seeds Ethyl Acetate Extract*
*Sample 2: GA : Guava seeds Alcohol Extract*

**FIG.11. ANTI DIABETIC ACTIVITY**




## RESULTS AND DISCUSSION

Guava seed was extracted with three solvents viz petroleum ether, ethyl acetate and alcohol 80%. After identification of phytoconstituents present in all the total extracts by preliminary phyto chemical analysis, they were further evaluated by chromatographic analysis. Thus these extracts were subjected to paper and thin layer chromatographic separation of their constituents. They confirmed the presence of various anthocyanidine, sugars and amino acids. Numbers of spots obtained were measured for Rf value calculation and the results are tabulated. They indicate the presence of different anthocyanidine, sugars, and amino acids in the single plant sample- the seed.

Anti diabetic activity of total extracts of ethyl acetate and alcohol 80% are evaluated by in vitro α amylase assay. The ethyl acetate extract showed better anti diabetic activity than even the standard at higher concentration of sample. Though the anti diabetic potential of alcohol (80%) extract is less than that of standard at all concentrations, that potential of ethyl acetate extract is significant at higher concentrations. Even at 500 µg concentration the percentage inhibition of ethyl acetate extract exceeds that of standard. It is confirmed at 1000 µg concentration of it.

## CONCLUSION

Ethyl acetate extract of the seeds of Psidium guajava.Linn has anti diabetic activity at higher doses, though the alcohol 80% extract was not promising. The constituent responsible for this activity can be isolated, identified and confirmed for this potential. Work towards that is underway in our laboratory.

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