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STUDY ON EXTRACTION, ISOLATION AND PHYSICO-CHEMICAL PROPERTIES OF PSIDIUM GUAJAVA

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Abstract
The guava (*Psidiumguajava*) belongs to family Myrtaceae and is one of the most gregarious of fruit trees. Most of the studieshave been conducted on the phytochemical, biological, and pharmacological of this plant and its parts. However, a very limited number of studies was reported on the physic-chemical properties of guava leaves extracts. The physical properties such as – pH, Solubility, Conductivity, Density and Viscosity, and Optical rotation, were examined and moisture and ash percentage also determined and all the mineral elements (calcium, magnesium, sodium and potassium) were also determined.The results had shown the greatest diversity in all the physical properties. The highest values were recorded with the sample C in case of all the physical parameters, except pH. The results of chemical properties was indicated of various samples contains the cations that is calcium, magnesium, potassium and sodium. The various aqueous extracts were subjected to TLC examination.

Introduction
India is a very richly populated country by various type of vegetation –plants; out of these a lot of plants are likely to become a great supplementary value for human being in the form of important drugs. Guava(*Psidiumguajava*- one of the most gregarious of fruit trees) is one ofsuch plants in folk medicine has been used for themanagement of various disease like malaria, vomiting, gastroenteritis, diarrhoea, wounds, dysentery, ulcers, coughs, toothache, inflamed gums, sore throat, and a number of other conditions and is believed to active(Purseglove 1968; Morton 1987;Lutterodt 1989; Jaiarj*et al* 1999; Abdelrahim*et al* 2002;).New leads/hits indrug discoveryhave been developed between natural sources and scientific link as folkloric medicinal use of some of these naturalproducts, especially plant's origin, to biologicalactivity. Therefor plants continue to provide a goodsource for new drugs (Kunle and Egharevba 2009;Kunle*et al* 2003; Begum *et al* 2002). Many workers have done a great amount of work on the medicinally important plants and have isolated various compounds and screened them for different types of biologically activities. The main driving force behind drugs research is the possibility of discovering new drugs and new modes of treatment for human ailents (Wilson 1980).

For the present work, I choose the leaves of Guava (*Psidiumguajava)* commonly called as Amrud belongs to family Myrtaceae. In vedic literature its name has been given as amrood (Amar and uder) which means a kind of amrit in all stomach trouble (Usher 1984; Prakesh 1972; Sharma 1998;Chopra 1956, and Nadkarni 1954). This plant is common throughout the world especially all worm areas of tropical America, West Indies, Asia and Africa, and the other subtropical countries including India. It was introduced in India by Portuguese. Themorphology of the plant has been described severallyin literature (Burkill 1997; Irvine 1961; Anas*et al*2008). The leaves and bark of the guava tree have a long history of use for medicinal purposes and are still employed today for the same. The plant is used for prevention and treatment of scruvy in Asia and Africa (Watt and Breanehwizk 1969: Council of Scientific and Industrial Research, 1969). Guava juice has been reported to possess hypoglycemic activity in both mice and humans (Cheng 1983).

In addition, guava leaves have been used to treat many ailments including caught and pulmonary diseases in Bolivia and Egypt (James 1950, and Batick 1984). Young guava leaves are used in India as a remedy against cough (Tiwari et al., 1974). People of China use guava leaves as a anti-inflammatory and haemostatic agent (Hon-Ning 1988). A decocation of leaves is used for the treatment of cholera (Usher 1984; Prakesh 1972; Sharma 1998; Chopra 1956; and Nadkarni 1954). The leaves of this plant have been employed for dying, tanning. The investigations have shown the presence of waxes, resins, sugars and essential oils (Hawkes 1983). Major number of studies has been conducted on the phytochemical, biological, and pharmacological of this plant and its parts. But a very limited study was conducted on the physical chemical properties of plant leaves extract. So the present study aim is to conduct the physicochemical properties of guava leaves extract.

INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

Materials and methods

Collection of plant leaves

Guava (*P.guajava*- 5 kg) leaves were collected from the garden of Sandipani Hostel, Vikram University campus, Ujjain. The leaves were cleaned, washed shaded dried. The shade dried leaves were made powder with the help of mechanical grinder and extracted.

Preparation of extracts from plant material - Guava leaves (*P. guajava*)

The leaves powdered (10 gm) were boiled with water in round bottom flask (150 ml) at different temperatures (100^0C, 110^0C, and 120^0C), until it reduced on half (~75 ml) of itsinitial volume. The extracts were cooled and filtered (Whatman No.1 filterpaper used for filtering). The time taken for boiling and final volume of the extract was recorded. The three samples (A,B,and C) were boiled at temperature 100^0C, 110^0C, and 120^0C to yield A, B, and C extracts respectively. All the three samples were obtained is good quantity so these were used further determination of physicochemical properties and Thin layer chromatography (TLC) examination of plant material.

Determination of physical properties

The physical properties such as – pH, Solubility, Conductivity, Density and Viscosity, and Optical rotation, wereexamined and moisture and ash percentage also determined. A digital pH meter (Systronics model no. 335) was used to determination of pH, whereas the solubility was determined with hexane, benzene, ether, ethyl acetate, chloroform, acetone, ethyl alcohol and methyl alcohol. A direct reading digital conductivity meter (Systronics model no. 304) and dipping type conductivity cell (cell constant $0.90cm^{-1}$) with platinized electrode were used for measuring the conductance of all the samples.For the determination of λ_{max}a micro-computer based UV visible spectrophotometer (Systronics model no. 106) with 1.0 cm quartz cell was used for all the absorbance measurement of samples with respect to water. The density of the aqueous extracts of *P. guajava* was determined with a pyknometer and Ostwald's type viscometer was used for measuring the viscosity of the samples. The viscosities of the samples (A, B, and C) were calculated by the using of following equation

$$\frac{\eta_1}{\eta_2} = \frac{\rho_1 t_1}{\rho_2 t_2}$$

Where $\eta_1\eta_2\rho_1$ $\rho_2$and t_1, t_2 are the viscosity, density and time flow of liquid (unknown)and known liquid respectively. All the measurements were made at room temperature. A polarrimeter (Schimadzu model no. C-170) consists of a polarizer, a cell to contain the samples and an analyzer (a second polarizing prism) that can be rotated sa as to compensate for the rotation induced by the samples was used for the measurement of optical rotation of all the samples, with respect to water. The moisture content of the extracts was estimate by **Pearson…** method (5.0 ml of extract was heated in a disk for the six hours in oven at 105^0C. it was cooled in deshisicator and weighted, the loss in weight was recored and the moisture content was calculated as follows)

$$\text{Percentage of moisture content} = \frac{100(p-a)}{p}$$

Whereas a= weight of ash (gm)

P= weight of sample (gm)

The ash contact of various extracts was determined by standard procedure of AOAC. 5.0 ml of the extract was weighted in a crucible which was ignited first gently over a small flame until thoroughly charred and then heated in a burnar until a ash was obtained the crucible was cooled in air and then in dessicator and weighed. The weight of the ash per 100 gm. Of extract was then calculated. The ash content was determined as follows

$$\text{Ash Content Percentage} = \frac{100 \times a}{p}$$

Whereas a= weight of ash (gm)

P= weight of sample (gm)

Chemical properties- quantitative estimation of mineral elements

For the estimation of mineral elements, the ash of the various extracts was prepared by hearting the extracts (15 ml) in a crucible oven, Bunsen burner. The ash was dissolved in hot dilute hydrochloric acid (40 ml), digested oven a hot waterbath, diluted and filtered through Whatmann No. 42 filter paper into a standard flask. The filter paper was washed several times with hot dilute hydrochloric acid. The volume was then made upon 250 ml. and this solution was used for the estimation of calcium, magnesium, sodium and potassium. All the mineral elements (calcium, magnesium, sodium and potassium) were determined by the gravimetric methods.

INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

TLC examination

After determination of the physical properties, the various extracts samples (A, B, and C) were subjected to TLC examination. The purity of the compounds were tested and confirmed by TLC examinations. The TLC was performed on silica gel G the chromatograms were developed either in iodine chamber or sprayed by concentrated H_2SO_4, followed by heating in an oven, at 100^0C for an hour. Silica gel (60-120 mesh) was used for column chromatography.

Results and Conclusion

Physical properties

Results (Physical and Chemical properties)of the extracts of the leaf of *P.guajava*are shown on Figure 1,2 and Tables 1, 2 and 3. The pH values from 4.72 to 5.02. Figure 1A shown the average values with stander deviation of all the samples. pH of the samples was decreased with increase the temperature. Whereas, the values of conductivity had increased regularly from first to last samples (Figure 1B). The increase in conductance with the increase in concentration may be due to the ionization. The weight of the ash per 100 gm. of extract was then calculated as per the formula explain earlier in materials and methods. The results were shown in figure 1C. The percentage of moisture content was recorded and the results are presented in figure 1D. The values of moisture percentage were in between 17.13 – 19.17. For density and viscosity, all the measurement was made at room temperature. The values of density and viscosity was vary from sample to samples maximum values for both was recorded in sample-C, whereas minimum for Sample-A (Figure Eand F). The results wereindicating the values of Optical rotation and Absorbance ranging between 6.50 -7.07 and 360-390 (figure G and H).

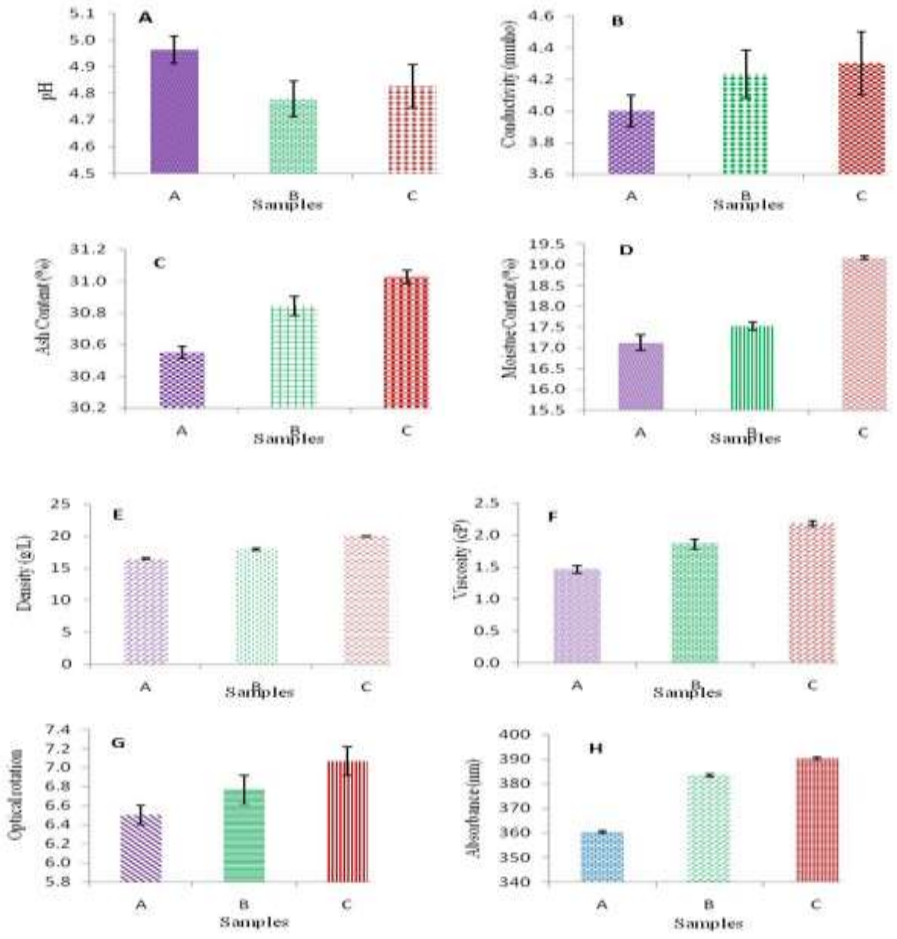

Figure 1 Physical property of Guava leaves (P. guajava); (A) pH, (B) Conductivity, (C) Ash content, (D) Moisture content, (E) Density, (F) Viscosity, and (G) Optical rotation (H) Absorbance.

Table 1 has shown the results of the solubility of the samples in various solvents. The results indicate that Ether, Acetone, Ethyl alcohol, and Methyl alcohol was soluble, while Hexane, Benzene, and Ester insoluble to all the samples. However, Chloroform was partial – soluble.

INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

Table 1 The solubility of the samples in various solvents

Solvents	**Sample A**	**Sample B**	**Sample C**
Hexane	Insoluble	Insoluble	Insoluble
Benzene	Insoluble	Insoluble	Insoluble
Ether	Soluble	Soluble	Soluble
Ester	Insoluble	Insoluble	Insoluble
Acetone	Soluble	Soluble	Soluble
Chloroform	Partial-soluble	Partial-soluble	Partial-soluble
Ethyl alcohol	Soluble	Soluble	Soluble
Methyl alcohol	Soluble	Soluble	Soluble

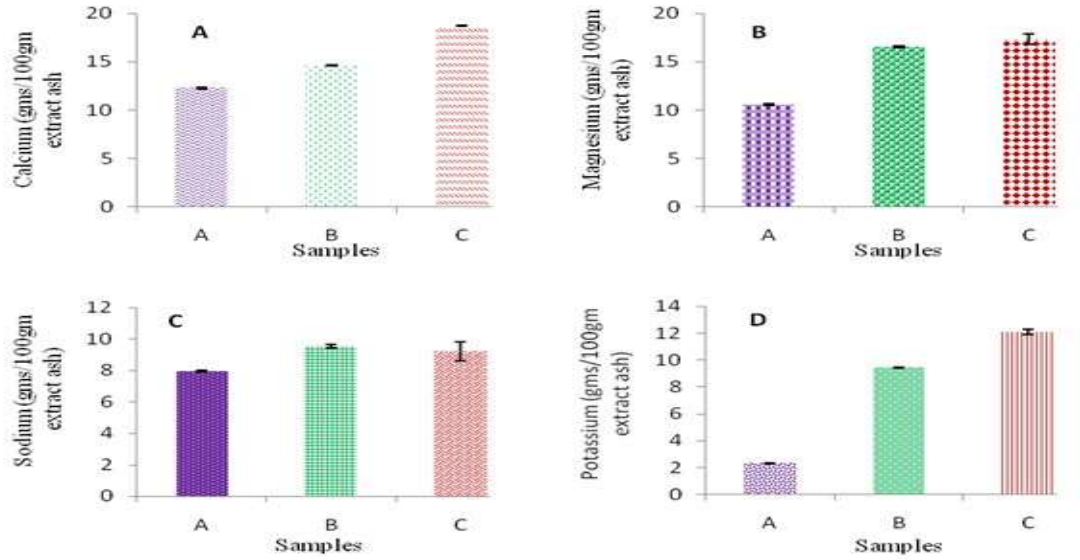

Figure 2Chemical propertiesof Guava leaves (P. guajava)- quantitative estimation of mineral elements; (A) Calcium, (B) Magnesium, (C) Sodium, and (D) Potassium

Chemical properties- mineral elements

The results indicated that various samples contain the cationsi.e calcium, Magnesium, Potassium, and Sodium. The percentage of calcium was then calculated by using the following formula (1ml of 0.05N $Kmno_4$ = 1 mg of calcium). The value of calcium was recorded between 14-18 gms/100gm extract ash (Figure 2A). However, the percentage of magnesium was calculated by multiplying the weight of the precipitate by a factor 0.218. The results indicated that the maximum value of sample-C and minimum for sample –A (figure 2B). Sodium and Potassium both the estimated as perchlorates. There was not similarities recorded between the samples for both the elements, for sodium maximum values was recorded for Sample-B, while for potassium it was for sample-C (Figure 2C and D).

TLC examination

The TLC examination with various solvents and their mixture indicated that they contained a large number of compounds. The results are presented inn Table 2. Evaporated the various extracts on water bath and coated on silica gel, dried and then extracted serially with benzene, ester, acetone, n-butanol, ethyl alcohol and methanol for 2-3 days, and were subjected to TLC examination with various solvent systems in increasing order of polarity. The results was recorded and presented in table 3.

Table 2: TLC examination with various solvents

Solvent Used Serially	**Amount of Solvents**	**Spot on TLC**
Hexane	10	No Spot
Hexane: Benzene	9:1	No Spot

INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

Hexane: Benzene: acetic acid	9 : 1 : 1 8 : 2 : 1	No Spot No Spot
Hexane: Benzene: Ether: Acetic acid	8 : 1: 1 : 0.5	No Spot
Chloroform : Water : Methanol	6 : 3 : 1	No Spot
Butanol : Water : Acetic acid	5 : 5 : 1 9 : 1 : 1 8 : 2 : 1	8-10 Spots

Table 3: TLC examination with various solvent synthesis in increasing order of polarity

Contents	**Solvent used serially**	**Ratios of solvents**	**Sports o TLC**
Benzene extract	Benzene : MeOH : AcOH	7 : 2 : 1	No Spot
Ethyl acetate extract	C_6H_6 : MeOH : H_2O	7 : 2 : 1	Single Spot
Acetone	C_6H_6 : MeOH : H_2O	7 : 2 : 1	Three Spot
n-Butanol	C_6H_6 : MeOH : H_2O	7 : 2 : 1	No Spot
Ethyl alcohol	C_6H_6 : MeOH : H_2O	7 : 2 : 1	Single Spot
Methanol	C_6H_6 : MeOH : H_2O	7 : 2 : 1	No Spot

Conclusions

The results had shown the greatest diversity in all the physical properties. The highest values were recorded with the sample C in case of all the physical parameters, except pH. The results of chemical properties was indicated of various samples contains the cations that is calcium, magnesium, potassium and sodium. The various aqueous extracts were subjected to TLC examination. These observations revealed the presence of a mixture of a large number of compounds. Elution was carried out with increasing order of polarity of solvents and their mixtures. The results were given in figure 1H. The entire three decoctions were reduced to small volume and coated on silica gel, and dried. The silica gel was then extracted with benzene, ethyl acetate, acetone, n-butanol, ethylalcohol and methanol, different fractions were collected and examined by TLC using solvent systems benzene: methanol: acetic acid and benzene : methanol : water.

The fractions collected from benzene gave no sport on TLC. The fraction collected from ethyl acetable gave a single sport with benzene : methanol : water. The fraction collected from acetone gave three spots with benzene : methanol : water. The fraction collected from ethyl alcohol gave several spots. There were no results of fractions collected from n-butanol and methanol with benzene : methanol : acetic acid and benzene : methanol : water. They perform various functions in nature and many of them have medicinal properties and interesting biological activity. They were also wide spread use in various systems of herbal medicine, including Ayurvedicmedicine.

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INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

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