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STUDIES OF MEDICINAL VALUES OF THE COMPONENTS PRESENT IN PSIDIUM GUAJAVA (P GUAJAVA) LEAVES

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Abstract— **Our present study on Psidium guajava to study phytochemical compounds present in its leaves and the medicinal value of the compounds. The P.guajava is an angiospermic plant belonging to the family Myrtaceae and it contains 150 genera and 3300 species. From the extraction data we conclude that we get the maximum percentage of extraction (crude extract) by methanol solvent which was about 7.3%. .Preliminary phytochemical studies showed maximum phytochemical compounds such as steroids , glycosides , phenolic compounds , tannins , carbohydrates , Saponins in the methanol extract. The qualitative phytochemical analysis of P.guajava leaf extract such as UV-Vis spectrum of crude extract of different solvents shows that absorption occurs at the visible region, thus we can use this compound as a photocatalyst in photocatalysis reactions. FTIR was used to identify the characteristic functional group in the crude Guava leaves powder .The absorption at 3425cm^{-1} is due to presence of hydroxyl group. The absorption at 1634 cm-1 is due to c=c stretching .The absorption at 1321 cm^{-1} is due to bending vibrations of C-H bonds of the methyl group. A strong and broad absorption band in the range 3000 − 3700 cm^{-1}, corresponding to the hydroxyl groups (O–H) stretching vibrations. This indicates the presence of alcoholic and phenolic groups with a wide variety of hydrogen bonding . Sharp peak at 2855 cm^{-1} and 2924 cm^{-1} associated with –C–H stretching vibrations of −CH2 and –CH groups, indicating the presence of aromatic ring (Ar–H) and glucose moieties (CH2OH) in tannins. The broad band in range 1200 − 1420 cm^{-1} and 764 − 820 cm^{-1} assigned to O– H in plane and out of plane blending, respectively . The sharp peak at 1604 cm^{-1} is attributed to the C=C stretching vibration in the phenolic groups. A small peak at 1700 cm^{-1} assigned to the carbonyl (–C=O) group indicates that an ester bond is formed between two galloyl groups. The peak at 900 − 1200 cm^{-1} is assigned to the C–O stretching vibration of phenolic groups. These phytochemical compounds can help in the pharmaceutical field.**

Keywords— **Guava (Psidium guajava), Phytochemical compounds, Soxhlet, TLC, UV-VIS, FTIR, GCMS,etc.**

I. INTRODUCTION

Mother nature blessed us with everything we need like food, shelter, medicine (as a phytochemical), cloth etc. we as an intelligent creature in this globe have to study nature and use those existing products for fruitful purposes. In India people use plants as a medicine from ancient times in the Ayurvedic medicine system for the treatment of various diseases. Moreover due to the less offshoot of herbal medicine most probably it is the future of modern medicine. Furthermore, due to the presence of the wide range of precious flora and fauna, North-East India is one of the thirty six biodiversity hotspots of India. (**Sasidhama et al., 2010** and **Priya et al., 2011)**

The Psidium guajava tree is native to Mexico, Central America, and the Caribbean and then spread to tropical and subtropical regions of the Americas, Australia, and Asia **(Sharma, 1991)** Today P. guajava trees are being produced in India, Nigeria, Philippines, Southeast Asia, Pakistan, Bangladesh, Brazil, China, and Mexico (**Shaheen et al., 2000)** and the leaves can be found in specialty markets and online stores across the world. The Psidium guajava is an angiospermic plant which belongs to the family Myrtaceae and it contains 150 ganera and 3300 species **(Sharma, 1991).** P. guajava leaves are oblong to oval in shape and average 7-15 cm long and 3-5 cm wide. The leaves grow in an opposite arrangement, which means two leaves grow at the same point on either side of the stem, and have short petioles, or stalks that join the leaf to the stem. The surface of the deep green P. guajava leaf is wide and leathery with faint white veins and some light brown patches. P. guajava leaves are aromatic when crushed and have a scent similar to that of the P guajava fruit. P. guajava leaves grow on a

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small tree with wide-spreading branches and copper-colored flaking bark that reveals a green base **(Sharma, 1991)**.

P guajava leaves have many anti-inflammatory properties and also contain vitamin C, vitamin B, antioxidants, and tannins. P guajava leaves are most popularly consumed in tea, as capsules, ground into pastes, and extracted as essential oils. Young leaves are traditionally preferred for medicinal benefits and can be found in health stores in various forms. They can also be found dried and ready for use in specialty tea stores. When dried, the leaves can be crushed and boiled to make the medicinal tea (**Jaiarj et al**., **1999).** The decoction or infusion of the leaves is used as febrifuge, antispasmodic and for rheumatism in India (**Shaheen et al., 2011)**. The leaves are used in the USA as an antibiotic in the form of poultice or decoction for wounds, ulcers and toothache. Bronchitis, asthma attacks, cough, pulmonary diseases could be also treated with P guajava teas (**Teixeira et al., 2003).**

The P guajava leaf extract has a highly antimicrobial activity, the water, alcohol and chloroform extract of fresh P guajava leaves has effective against Aeromonas hydrophila, Shigella spp. and Vibrio spp. Also, water extract of dried leaves possesses bacterial activity against Staphylococcus aureus, Sarcina lutea and Mycobacterium phlei. **(Pranee et al., 1999).** Beside that, P guajava leaves have quercetin-3-arabinoside and quercetin which can be isolated from leaves. Its leaves contain a compound which has morphine-like action and controls the muscular tone. Quercetin repressed intestinal contraction encouraged by 19 enhanced absorption of calcium. Quercetin has a strong effect on ileum. It is thought that quercetin in the P guajava leaf is responsible for its spasmolytic activity. P guajava has high cytotoxicity **(Teixeira et al., 2003)**. P guajava can be used to treat the diarrhea caused by the E.coli or S. aureus toxins **(Vieira et al., 2001).**

P guajava contains high amounts of antioxidants and anti-providing nutrients which are essential not only for life but also help to control the free radical activities. It also has a variety of phytochemicals which are beneficial for human health like diabetes, obesity and high blood Pressure. There are two common methods by which antioxidants neutralize free radicals : DPPH and FRAP assay. Extracts of P guajava in water and organic solvents have a large quantity of antioxidants which can stop the oxidation reaction. The concentration of these compounds become high with the increase in concentration **(Pharmacopoeia Commission, 2000).**

The topic of this project work is on a preliminary study about different chemical compounds present in a P guajava leaf extract and profiling of the compounds present targeting important compounds with known medicinal values or having prospecting medicinal value.

The study basically involves the following two parts:

1. Extraction of leaves of a plant species. The extraction process followed is mainly Soxhlet extraction with different solvents.
2. Separations of the compound present in the leaf extract by TLC, UV, and IR spectroscopy.

II. MATERIAL AND METHOD

2.1 Pre-treatment of raw material

The parts of the plant's leaves were thoroughly washed with water in order to remove dust and inorganic material or any unwanted material sticking to the samples. The samples were air dried for 4-10 days depending on the sample type i.e. leaves .The samples were not undergone oven dry as at high temperature volatile or heat-labile compounds may be lost. Grinding of samples are done using a metallic grinder and porcelain mortar pestle . Grinding gives smaller sample particles with smaller particle size increases surface leading to better contact between samples and extraction solvents . The weights of the grinded samples were taken in electronic weighing balance and recorded the weight of the total amount. From this total amount, the desired amount for extraction i.e.10 gram was weighed and taken separately for extraction. Remaining part was stored in an airtight plastic zipper packet. (**Vieira et al.,** 2001 and **Tonal et al.,** 1999)

2.2 Extraction methods:

2.2.A Soxhlet extraction

Extraction of dried and powdered plant samples are carried out by using the soxhlet extraction process, For which soxhlet Apparatus of 200ml capacity with flask of size 500ml (BOROSIL) is used.For extraction , methanol , chloroform, acetone, hexane (100- 110) is used as solvent . The sample (10 gm., weighed precisely in electronic balance) was placed in a porous thimble covered with cotton wool and then the thimble is placed in the inner tube of the soxhlet chamber of the apparatus and then fitted to a round bottom flask that contain 250 ml solvent i.e. .methanol .Allihn condenser (having inlet and outlet tubing for water supply from tap water) was fitted to the upper joint of the soxhlet apparatus. Heat was applied using heating mantle to heat the solvent to its boiling point . As the heating continued, the solvent in the flask started boiling within a few minutes of heating and the solvent began to evaporate through the soxhlet chamber and get condensed in the condenser. Then solvent drops start falling from the top to the sample in the thimble. When the solvent in the soxhlet chamber reached to the level of the top of the siphon head, the solvent siphoned into the flask and thus bring the solvent containing extracted fat into the flask and at the same time the cycle of evaporation of solvents, condensation, extraction, and siphoning back is simultaneously goes on .It was noticed that one cycle of this evaporation, condensation, filling up of the soxhlet chamber and simultaneous siphoning back of solvent happens in 10

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minutes. This extraction process was allowed to continue for 6 hours. **(Azwanida, 2015).**

2.2. B Extraction of bulk extracts:
After extraction, the bulk solution obtained is concentrated by evaporating the solution in rotatory evaporator. The rotatory evaporator is a rapid evaporation system which involves evaporation of solvents in temperatures lower than their boiling point under lower atmospheric pressure i.e. under vacuum. Under this vacuum rapid evaporation is achieved through rotation of the evaporating flask dipped in the heating bath (in 30-40◦C which is lower temperature than the boiling point of the solvent) and rapid condensation of the evaporated solvent over a chilled water circulating condensing coil. The rotatory evaporator Is of Make Buchi (Switzerland) and Model: Rl-250; V-700 with chiller from Polyscience (USA). The concentrated volume of Solution stored in a sample tube for further analysis **(Harborne, 1998).**

2.2.C Thin layer chromatographic studies:
Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel G as stationary phase.

2.3 Qualitative Phytochemical Analysis:
2.3.A UV-visible spectrum analysis
The extract was centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper. The sample was diluted to 1:10 with the same solvent . The extract was scanned at wavelengths ranging from 200 to 1100 nm using the Perkin Elmer spectrophotometer and the characteristic peaks were detected . The peak values of the UV-VISIBLE were recorded **(Harborne, 1998).**

2.3.B Fourier Transform Infrared Spectroscopy (FTIR):FTIR was used to identify the characteristic functional group in the crude extract of guava leaves powder. A small quantity (5mg) of the powder was dispersed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at pressure of 6 bars within 2 min. to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using the Perkin Elmer 2000 infrared spectrometer. The sample was scanned from 4000 to 400cm-1 16 times to increase the signal to noise ratio. (**Sharma, 2018**)

III. RESULTS AND DISCUSSIONS

3.1 Phytochemical Analysis of leaf extract
Phytochemical screening of methanol, hexane, chloroform, and acetone extracts revealed the presence of flavonoids, tannins, terpenoids, saponins, alkaloids, phenolic compounds and carbohydrates . Guava leaves have several chemical constituents such as comarins, essential oils, flavonoids, triterpenes and ellagitannins which are known to have antimicrobial properties. Polyphenolic compounds dominate Guava leaves are flavonoids (>1.4%) and tannins. Antibacterial compounds derived from plants could be phenolic substances such as flavonoids (**Tonal, 1999)** . Tannins can be obtained from almost any kind of green plants; however, the quantity varies. Tannins are polyphenolic compounds that are in plants, food and beverage (**Nakatani, 1988)** Guava leaves contain tannin by 9%, which can be used as an antibacterial. Tannins can be used as an antibacterial substance because it has a phenol group, so that the tannins have properties like alcohol is an antiseptic that can be used as an antimicrobial component (**Makkar and Becker, 1998)**

Sl No	Name of solvent	Amount of solvent taken	Amount of powdered P guajava leave (gm)	Amount of product extracted	Percentages of product extracted
1	Methanol	110	10	0.73	7.30%
2	Acetone	110	10	0.53	5.30%
3	Chloroform	110	10	0.502	5.02%
4	Hexane	110	10	0.5	5%

Table 1 Extraction data table :extraction of crude extract of Psidium guajava leaves L using different solvents.

From the extraction data table we conclude that we get the maximum percentage of extraction (crude extract) by methanol solvent which is about 7.3%.

3.2.Separations:
3.2.1.Thin layer chromatographic profiling

Sl No	TLC profile of solvent extract P. guajava leaf	Methanol : Hexane	Rf values
1	METHANOL	5:5 and 8:8	0.35, 0.84, 0.88, 0.95, 0.99
2	CHLOROFORM	02:08	0.57,0.94
3	ACETONE	02:08	0.41,0.6,0.75 ,0.90
4	HEXANE	0.274305556	0.97,1.0,1.5, 2.0

Table 2 Thin layer chromatographic profiling of crude extracts of leaves of P.guajava L.

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3.2.2 UV AND IR GRAPHS:

The UV-Vis spectrum of crude extract of Guava Leaves from the different solvents .

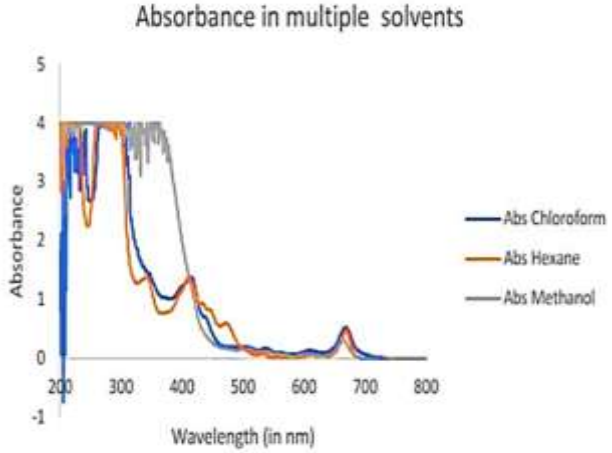

Fig 3.2.2.1 The UV-Vis spectrum of crude extract of Guava Leaves from the different solvents.

UV-Vis spectrum (Fig 3.2.2.1) shows that absorption occurs at the visible region, thus we can use this compound as a photocatalyst in photocatalysis reactions.

3.2.3 Fourier Transform Infrared Spectroscopy

The aim of FTIR analysis is to determine the existence of a functional group that exists on isolate. The IR spectrum of the crude powder of Guava leaves. The absorption at 3425cm^{-1} is due to the presence of hydroxyl group. The absorption at 1634 cm^{-1} is due to c=c stretching .The absorption at 1321 cm^{-1} is due to bending vibrations of C-H bonds of the methyl group. The infrared spectra of guava leaf extract show a strong and broad absorption band in the range 3000 − 3700 cm^{-1}, corresponding to the hydroxyl groups (O–H) stretching vibrations. This indicates the presence of alcoholic and phenolic groups with a wide variety of hydrogen bonding . sharp peak at 2855 cm^{-1} and 2924 cm^{-1} associated with –C–H stretching vibrations of −CH2 and –CH groups, indicating the presence of aromatic ring (Ar–H) and glucose moieties (CH2OH) in tannins. The broad band in range 1200 − 1420 cm^{-1} and 764 − 820 cm^{-1} assigned to O– H in plane and out of plane blending, respectively (**Falcao and Araujo,** 2013). The sharp peak at 1604 cm^{-1} is attributed to the C=C stretching vibration in the phenolic groups. A small peak at 1700 cm^{-1} assigned to the carbonyl (–C=O) group indicates that an ester bond is formed between two galloyl groups. The peak at 900 − 1200 cm^{-1} is assigned to the C–O stretching vibration of phenolic groups.

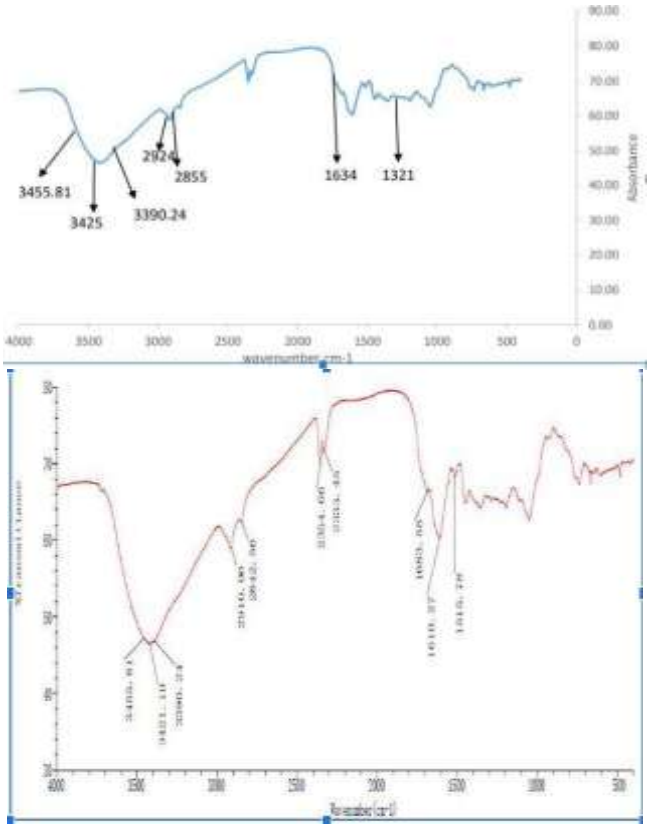

Fig :3.2.3.1 FTIR of methanol leaf extract of P.guajava

IV. CONCLUSION

We did our present study on Psidium guajava to study phytochemical compounds present in its leaves and the medicinal value of the compounds present in P.guajava leaves . The P.guajava is an angiospermic plant belongs to the family myrtaceae and it contains 150 genera and 3300 species (sharma 1991).From the extraction data table 1 we conclude that we get the maximum percentage of extraction by methanol solvent which was about 7.3% .Phytochemical screening of methanol, hexane, chloroform, and acetone extracts revealed the presence of flavonoids, tannins, terpenoids, saponins, alkaloids, phenolic compounds and carbohydrates .The qualitative phytochemical analysis was helpful in determining the electronic structure of unsaturated molecules and for measuring the extent of their conjugation and functional groups in P.guajava leaf extract .The UV-Vis spectrum of crude extract of Guava Leaves from the different solvents .UV-Vis spectrum (Fig 3.2.2.1) shows that absorption occurs at the visible region, thus we can use this compound as a photocatalyst in photocatalysis reactions.FTIR was used to identify the characteristic functional group in the crude Guava leaves powder.

Finally, we can conclude from this study that the phytochemical compounds like saponins, tannins, flavonoids, alkaloids, carbohydrates, terpenoids,and phenolic compounds were determined in methanol leaf

extract were present in P. guajava leaves which can be used in medicinal fields in anti-microbial ,anti-diarrheal, anti-inflammatory ,antioxidant activities.

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