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Phytochemical Analysis and FT-IR Fingerprinting of Pineapple Peel-A Natural Resource of Bioactive Compounds

A.S. Lubaina*[1], P.R. Renjith[1] and K.V. Dinesh Babu[2]
[1]Department of Botany, Christian College, Kattakada, Thiruvananthapuram, Kerala, India.
[2]Department of Chemistry, Government College for Women, Thiruvananthapuram, Kerala, India.

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***Corresponding Author Email:** lubainanizam@gmail.com

Abstract

Aims: Fruit wastes and their by-products are formed in great amounts during industrial processing and its disposal being one of the major problems faced by all parts of our state. The present study is an attempt to recycle pineapple peel - a byproduct of pineapple processing industries to study the qualitative and quantitative analysis of phytochemicals and FT- IR reflectance spectra of the petroleum ether, ethyl acetate, ethanol and aqueous extracts. **Materials and methods:** The four solvent extracts of pineapple peel were qualitatively screened for the presence of different phytochemicals followed by the quantification of phenols and flavonoids. Tablets for FT-IR spectroscopy were prepared in an agate mortars, by mixing extract powder with KBr and the absorbance spectra were measured between 400 and 4000/cm. **Results:** The qualitative phytochemical screening of the petroleum ether, ethyl acetate, ethanol and aqueous extracts of pineapple peel revealed the presence of phenols, flavonoids, alkaloids, tannins, saponins, terpenoids and steroids. The estimation of the total phenols and flavonoids showed an enhanced profile of phenols compared to flavonoids. The FT-IR spectrum exhibits the characteristic fingerprint banding features in all the extracts. Several indicator bands that are pertained to functional groups represent chemical components or metabolic products. Among the four extracts evaluated the ethyl acetate fraction showed significant amount of phytochemicals as well as IR fingerprinting pattern. **Conclusion:** The pineapple peel contained an appreciable amount of phytochemicals and bioactive compounds revealed it's potential for the utilization of pineapple waste in pharmaceutical as well as cosmetic industries.

Keywords
flavonoids, FT-IR, phenols, phytochemical screening, pineapple peel.

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INTRODUCTION

The use of plants and plant products could be traced as far back as the beginning of human civilization. The beneficial effects of plants mainly attributed to the phytochemical compounds that are capable of producing definite physiological action on body **[1]**. The naturally occurring phytochemicals offer promise to be used as safe alternatives. Although secondary plant metabolites provided numerous leads for the development to fan array of therapeutic drugs, the discovery of new drugs with novel structures has declined in the past few years. Studies of secondary metabolites from plants reveal many original compounds that are useful to mankind. Chemical compounds from plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs, thus herbal medicines do not differ greatly from conventional medicine in terms of how they work. Some phytochemicals bind physically to cell wall and thereby prevents the adhesion of pathogen to human cell wall. This enables herbal medicines a great demand in the developed and developing countries for primary health.

Fourier Transform – Infrared spectra (FT-IR) promises to be of a great value because of its sensitivity, rapidity low expense and simplicity. This together with the large information already known about spectral peaks obtained from FTIR spectra of living cells make FTIR spectroscopy as an attractive technique for detection and identification of phytochemicals. One of the important applications of the infrared spectroscopic study is the diagnostic value in establishing the presence of certain organic constituents in plants. FT-IR spectroscopy provides more detailed chemical information on the samples composition because it measures the fundamental vibration used as a metabolic fingerprinting tool in plants.

Pineapple being one of the leading tropical fruits produces large quantities of solid and liquid wastes. In the case of pineapple fruit 60% of the fruit weight was discarded as waste in the form of peel, core, crown and stem and its disposal being one of the major environmental problems faced by our state and causing pollution. A high level of BOD and COD in pineapple wastes add to further difficulties in disposal. Pineapple waste could be a potential source for the extraction of beneficial bioactive compounds might have a potential for recycling to get raw material or for conversion into higher value added products or even as a raw material for other industries for use as food or feed after biological treatment **[2]**. Natural products from plants may offer several new agents for various industries. Phytochemicals from fruit peels have provided a source of inspiration for several physiological processes on humans. In this scenario, the present study aims to evaluate the qualitative and quantitative analysis of phytochemicals and FT-IR fingerprinting using petroleum ether, ethyl acetate, ethanol and aqueous extracts of pineapple peel which in turn providing an informative result for future research leading to the isolation of new and novel compounds from pineapple peel.

MATERIALS AND METHODS

Plant material

The material used for the study was the peel of Mauritius variety (the most popular cultivar grown in Kerala) of pineapple fruit collected from fruit processing industry at Vazhakulam, Muvattupuzha, which is the main cultivation region of pineapple in Kerala.

Soxhlet hot continuous extraction

250 g pineapple peel were finely chopped, air dried in shade at room temperature, powdered and successively extracted with 100 ml of petroleum ether, ethyl acetate, ethanol and water for eight hours using soxhlet hot continuous extraction method. The extracts were filtered and concentrated using rotary evaporator at 50°C. The yields of extracts were calculated.

Preliminary Qualitative Phytochemical Screening

The four solvent extracts (petroleum ether, ethyl acetate, ethanol and water) of pineapple peel from the soxhlet hot continuous method were screened for the presence of different phytochemicals based on the method of **[3]**.

Detection of Alkaloids

Wagner's test: Wagner's reagent (1.27g of iodine and 2g of potassium iodide dissolved in 5 ml of water and made up to 100 ml with distilled water) added to a fraction of the extract and a reddish-brown precipitate confirms the presence of alkaloids.

Detection of Phenolic compounds

Ferric chloride test: To a fraction of the extract add Neutral ferric chloride (5 %) solution and the presence of a deep blue colour indicates phenolic compounds.

Detection of Flavonoids

Aqueous NaOH test: To an aliquot of the extract, add 1N aqueous NaOH, resulting yellow orange colour detects flavonoids.

Detection of Saponins

Foam test: A small quantity of the extract was vigorously shaken with water and formation of persistent foam detects saponins.

Detection of Tannins

Ferric chloride test: A small amount of the extract was diluted with distilled water in the ratio 1:4, add a few drops of 10 % ferric chloride solution and appearance of a blue or green colour indicates the presence of tannins.

Detection of Terpenoids

Liebermann-Burchard test: Extract (1ml) dissolved in chloroform and a few drops of acetic anhydride followed by the addition of a few drops of H2SO4 giving a dark green colour detects terpenoids.

Detection of steroids

To the extracts evaporated to dryness, add a few drops of acetic anhydride and concentrated H2SO4; an array of colour changes from yellow, green and brown to black indicates the presence of steroids.

Quantification of Phenols and Flavonoids

Total phenol content was estimated by the method of **[4]**. An aliquot of the petroleum ether, ethyl acetate, ethanol and aqueous extracts were pipetted out separately and made up to 3 ml with 80% methanol. 0.5 ml Folin-ciocalteau reagent was added and kept for 3 min. 2 ml of 20% Na2CO3 was added to the mixture and kept in boiling water bath for 1 min. The white precipitate was removed by centrifuging it for few min and the absorbance of the clear blue solution was recorded at 650 nm against the blank containing 3 ml of 80% methanol, 0.5 ml Folin's reagent and 2 ml of 20% Na2CO3. The reaction between phenols and an oxidizing agent phosphomolybdate in Folin-ciocalteau reagent resulted in the formation of a blue complex. A standard graph of phenols was constructed with pyrocatechol by taking absorbance against concentration. The total phenols/g tissue was calculated from the standard graph.

The total flavonoid content of the petroleum ether, ethyl acetate, ethanol and aqueous extracts were determined by AlCl3 method with slight modification. Briefly 100 µl of the extract was mixed with 100 µl of 20% AlCl3 and 2 drops of glacial acetic acid. The mixture was diluted with methanol to 3 ml. After 45 min, the OD was read at 415 nm using the extract without AlCl3 as blank. Standard curve was made using quercetin (50-250 µg/ml) in methanol under the same condition. Total flavonoids were expressed as mg quercetin equivalent/g of weight (Absorbance = 4.9747 mg quercetin, R2= 0.9846) **[5]**.

Fourier Transform Infrared (FT-IR) Spectrophotometer

Fourier Transform Infrared (FTIR) Spectrophotometer is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of different solvent extracts of each plant materials was used for FTIR analysis. 2 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FT-IR spectroscope (Shimadzu, IR Affinity 1, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}

Statistical analysis

The data was statistically analyzed by one-way analysis of variance (ANOVA) and t-test ($p < 0.05$). The results are average of five replications and were represented as mean ± SD.

RESULTS AND DISCUSSION

Phytochemical screening of the petroleum ether, ethyl acetate, ethanol and water extracts of pineapple peel provides an understanding of the nature of metabolites present in them. All the extracts showed marked variability in the amount of phytochemicals. The qualitative screening of various phytochemicals in the four extracts revealed the presence of phenols, flavonoids, alkaloids, steroids, saponins, terpenoids and tannins (Table 1). The phenols and flavonoids are found in higher amounts in both ethyl acetate and ethanol extracts. The preliminary phytochemical tests are helpful in finding therapeutic phytochemical constituents in the plant material and also in locating the source of pharmacologically active chemical compounds. The therapeutic value of plants lies in its principle component which play potential physiological role on humans.

Further the quantitative analysis of phenols and flavonoids were carried out using all the four extracts of pineapple peel showed marked variability in the amount of phytochemicals (Table 2). The ethyl acetate extract of pineapple peel showed highest total phenol and flavonoid content followed by ethanol, water and petroleum ether extracts. The amount of total phenol and flavonoid from ethyl acetate extract were recorded as 74.92 ± 0.25 mg /g tissue and 32.5 ± 0.63 mg /g tissue respectively. The

results of quantitative estimation of total phenols and flavonoids from petroleum ether, ethyl acetate, ethanol and aqueous extracts of pineapple peel were tabulated in Table 2. The estimation of the total phenols and flavonoids showed an enhanced profile of phenols compared to flavonoids. The quantitative estimation of phenols and flavonoids in pineapple peel gives an insight to their chemical nature which can provide a rich data in understanding certain basic pattern of growth and metabolism. At the same time phenols and flavonoids can be used as chemical markers in taxonomic studies **[6]**. The amount and composition of these secondary metabolites were observed to be diversified at cellular and sub cellular level within the waste material of a plant.

Plant polyphenols are most abundant secondary metabolites of plants that protects plants from oxidative stress i.e., they are having redox properties act as reducing agents, hydrogen donors and singlet oxygen quenchers have drawn increasing attention due to their crucial ability to act as antioxidants. **[7]**. Polyphenols also play an important role in giving protection to the plant against deleterious effects of UV rays **[8]**, and also against phytopathogenic organisms. The high phenolic concentrations observed in plants may contribute to its defense mechanism. The pharmacological value of plant extracts lies in some chemically active compounds that produce a definite physiological action on human body. The most important of these bioactive constituents of plants are alkaloids, flavonoids and phenolic compounds **[9]**.

FT-IR Analysis

Fourier Transform Infrared Spectroscopy (FT-IR) is a high-resolution analytical technique used to identify the bioactive chemical constituent and to reveal the structure of the compounds **[10]**. In Fourier Transform Infrared Spectroscopy, molecule shows absorption in a characteristic range of frequency. The organic compounds mainly absorbed in the range of 4000-400 cm^{-1} which exhibits characteristic fingerprint band features play a key role in the study of these compounds and used to identify the cellular components. The FT-IR spectrum of samples collected from petroleum ether, ethyl acetate, ethanol and aqueous extracts of pineapple peel are shown in Fig. 1, Fig. 2, Fig. 3 and Fig. 4. A summary of the absorption bands of all the four extracts are given in Table 3.

The petroleum ether extracts resulted absorption in 2924.09, 2852.76, 1724.36, 1598.99, 1460.11, 1371.39, 1246.02, 1139.93, 1089.78, 989.48 and 358.76 cm^{-1} (Table 3) (Fig. 1) whereas the ethyl acetate extract started from 3460.30, 3404.36, 3284.77, 3257.77, 3226.91, 3161.33, 3147.83, 3111.18, 3049.46, 2939.52, 2887.44, 2360.87, 2333.87, 1726.29, 1593.20, 1325.10, 1301.95, 1253.73, 1064.71, 1002.98, 819.75, 678.94, 650.01 and 565.14 cm^{-1} (Table 3) (Fig. 2). The ethanol extracts of pineapple peel exhibited characteristic band pattern in the range of 3005.10, 2927.94, 2850.79, 2729.27, 2673.34, 1735.93, 1710.86, 1454.33, 1377.17, 1365.60, 1352.10, 1240.23, 1220.94, 1170.79, 1097.50, 1031.92, 968.27, 920.05, 889.18, 866.04, 842.89, 719.45, 684.73, and 358.76 cm^{-1} (Table 3) (Fig. 3). The water extract revealed absorption bands at 3290.56, 3226.91, 3149.76, 3136.25, 3113.11, 3095.75, 3082.25, 3051.39, 3014.74, 2881.65, 1597.06, 1323.17,1307.74, 1251.80, 1074.35, 999.13, 680.87, 653.87 and 351.04 cm^{-1} (Table 3) (Fig. 4). The highest number of peaks were noticed in the ethyl acetate and ethanol extracts (24 numbers) followed by aqueous extract (19 numbers) and petroleum ether (11 numbers) respectively. It was found that ethyl acetate and ethanol extract of pineapple peel provide more bioactive molecules than aqueous and petroleum ether extracts.

The signals between 3460.30-3111.18 cm^{-1} refers to stretching vibrations of OH groups like alcohols and phenols. The peaks between 2927.94 and 2850.79 cm^{-1} indicates the presence of alkanes whereas absorption at 3005.1 cm^{-1} and 3049.46 cm-1 corresponds to asymmetric stretching vibrations of alkenes. The signals at 2924.09 and 2852.76 cm-1 (hydrogen bond OH stretch) and at 1724.36 (C=O stretch) refers to the presence of carboxylic acid. The absorption at 2729.27 cm^{-1} corresponds to the stretching vibrations of aldehydes, 1724.36 cm^{-1} to ketones and 1593.20 cm^{-1} to amides respectively. A peak at 1597.06 cm^{-1} (N=O stretch), 1323.17 cm^{-1} and 1307.74 cm^{-1} (N=O bend) corresponds to the stretching and bending vibrations of nitro group. The signals at 1454.33 cm^{-1} and 1460.11 cm^{-1} indicates the bending vibration of N-H bend of secondary amines. The peaks between 1246.02 - 1089.78 cm^{-1} indicate the stretching vibrations of esters and ethers (C–O stretch). Absorption below 1000 cm^{-1} corresponds to C-H bending vibrations of isoprenoids **[11]**. The FT-IR analysis of pineapple peel confirmed the presence of alcohols, phenols, alkanes, alkenes, carboxylic acids, aldehydes, ketones, amides, esters, ethers, secondary amines and nitro compounds. The infrared spectrum is able to identify not only the major components in organic materials, but also to find some differences among them. FT-IR spectrum reflecting the panorama of chemical constituents in a complex system is the most credible method to

validate and identify the compounds used in traditional and herbal medicine **[12]**.

Table 1. Preliminary phytochemical analysis using petroleum ether, ethyl acetate, ethanol and water extracts of pineapple peel

Phytochemicals	Petroleum ether	Ethyl Acetate	Ethanol	Water
Alkaloids	++	+++	++	+
Saponins	-	++	-	++
Tannins	-	++	+	++
Phenols	++	+++	+++	++
Flavonoid	+	+++	+++	++
Terpenoids	+++	++	+	-
Steroids	+	+++	++	+

Strong positive +++; Moderately positive ++; Low positive +; negative test -.

Table 2. Total phenols and flavonoids of petroleum ether, ethyl acetate, ethanol and water extracts from pineapple peel. Values are mean ± SD of three independent replications

Solvent	Phenol (mg/g tissue)	Flavonoids (mg/g tissue)
Petroleum ether	11.1 ± 0.38	08.7 ± 0.09
Ethyl acetate	74.9 ± 0.38	32.5 ± 0.63
Ethanol	66.03 ± 0.14	20.6 ± 0.14
Water	18.8 ± 0.79	10.6 ± 0.07

Table 3. FT- IR Spectral analysis of petroleum ether, ethyl acetate, ethanol and aqueous extracts of pineapple peel

Frequency (cm^{-1})	Petroleum ether	Ethyl acetate	Ethanol	Aqueous
351.04	-	-	-	-
358.76	+	-	+	-
565.14	-	+	-	-
650.01	-	+	-	-
653.87	-	-	-	+
678.94	-	+	-	-
680.87	-	-	-	+
684.73	-	-	+	-
719.45	-	-	+	-
819.75	-	+	-	-
842.89	-	-	+	-
866.04	-	-	+	-
889.18	-	-	+	-
920.05	-	-	+	-
968.27	-	-	+	-
989.48	+	-	-	-
999.13	-	-	-	+
1002.98	-	+	-	-
1031.92	-	-	+	-
1064.71	-	+	-	-
1074.35	-	-	-	+
1089.78	+	-	-	-
1097.50	-	-	+	-
1139.93	+	-	-	-
1170.79	-	-	+	-
1220.94	-	-	+	-
1240.23	-	-	+	-
1246.02	+	-	-	-

1251.80	-	-	-	+
1253.73	-	+	-	-
1301.95	-	+	-	-
1307.74	-	-	-	+
1323.17	-	-	-	+
1325.10	-	+	-	-
1352.10	-	-	+	-
1365.60	-	-	+	-
1371.39	+	-	-	-
1377.17	-	-	+	-
1454.33	-	-	+	-
1460.11	+	-	-	-
1593.20	-	+	-	-
1597.06	-	-	-	+
1598.99	+	-	-	-
1710.86	-	-	+	-
1724.36	+	-	-	-
1726.29	-	+	-	-
1735.93	-	-	+	-
2333.87	-	+	-	-
2360.87	-	+	-	-
2673.34	-	-	+	-
2729.27	-	-	+	-
2850.79	-	-	+	-
2852.72	+	-	-	-
2881.65	-	-	-	+
2887.44	-	+	-	-
2924.09	+	-	-	-
2927.94	-	-	+	-
2939.52	-	+	-	-
3005.10	-	-	+	-
3014.74	-	-	-	+
3049.46	-	+	-	-
3051.39	-	-	-	+
3082.25	-	-	-	+
3095.75	-	-	-	+
3111.18	-	+	-	-
3113.11	-	-	-	+
3136.25	-	-	-	+
3147.83	-	+	-	-
3149.76	-	-	-	+
3161.33	-	+	-	-
3226.91	-	+	-	+
3257.77	-	+	-	-
3284.77	-	+	-	-
3290.56	-	-	-	+
3404.36	-	+	-	-
3460.30	-	+	-	-

Fig 1. FT-IR finger printing of petroleum ether extract of pineapple peel

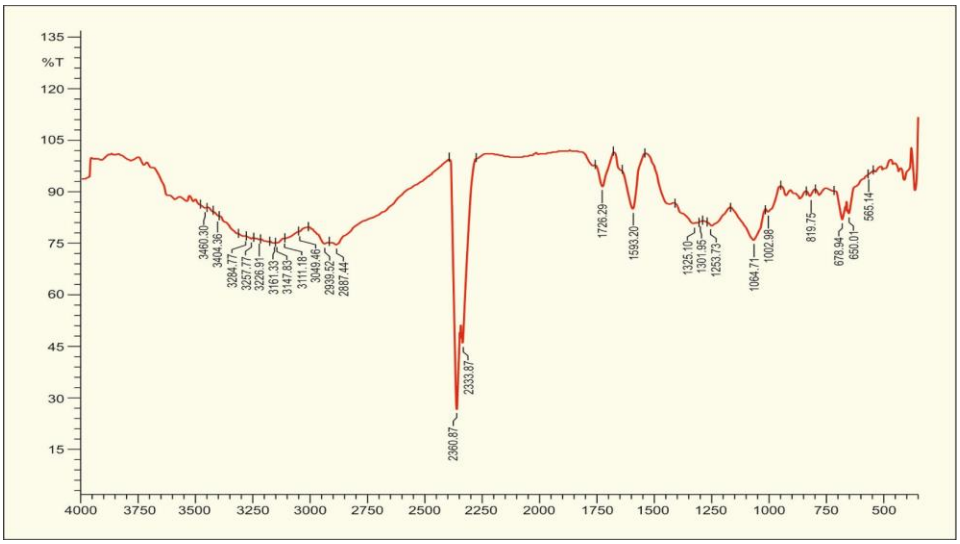

Fig 2. FT-IR finger printing of ethyl acetate extract of pineapple peel

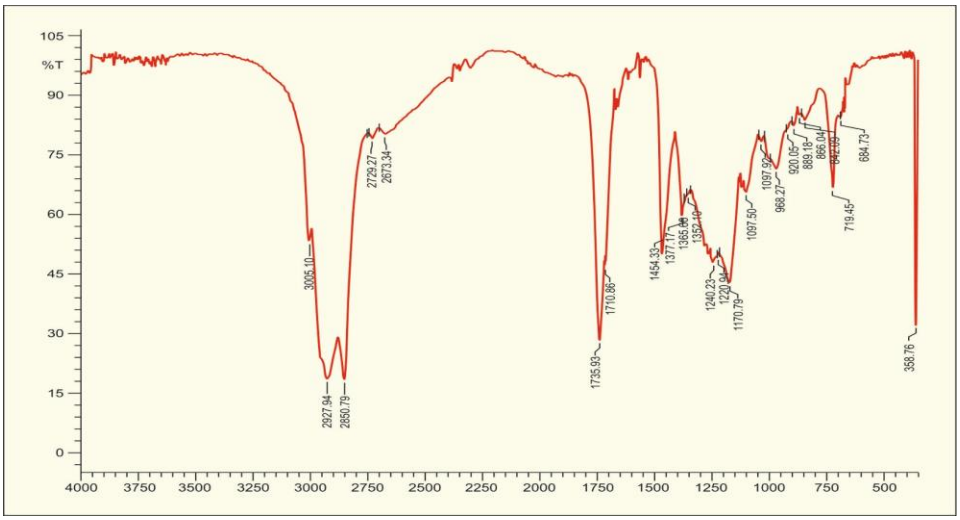

Fig. 3. FT-IR finger printing of ethanol extract of pineapple peel

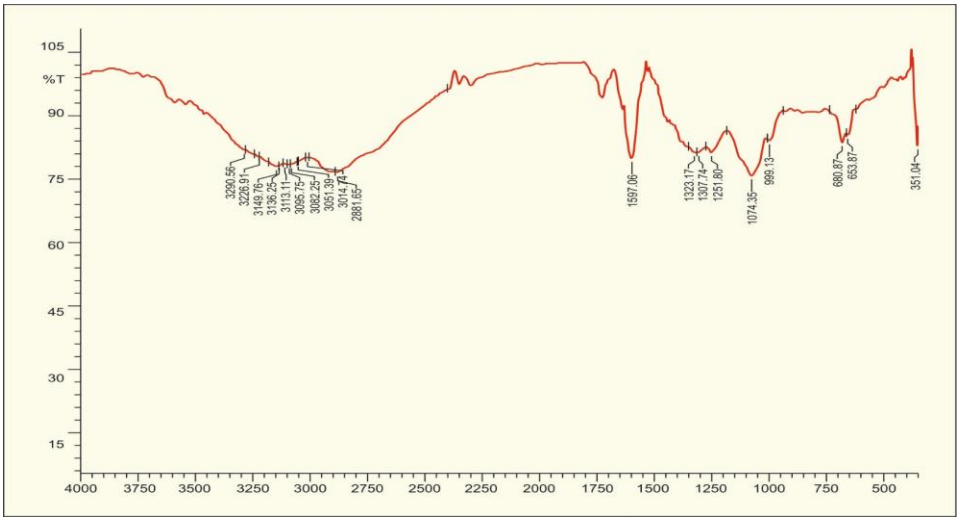

Fig 4. FT-IR finger printing of aqueous extract of pineapple peel

CONCLUSION

For the last decade, efforts have been made in the identification and isolation of bioactive phytochemicals from fruits wastes for re-using it with immense value to human life. The results of this study revealed that pineapple peels contain an array of phytochemicals such as phenols, flavonoids, alkaloids, saponins, tannins, steroids and terpenoids. The quantitative estimation of major secondary metabolites such as phenols and flavonoids also showed a remarkable level in all the four extracts. Flavonoids and phenolic acids are the most important group of secondary metabolites in fruits and are the good source of natural antioxidants capable of scavenging free radicals thereby protects the biological system against the harmful effects of oxidative process on macromolecules such as carbohydrate, proteins, lipids and DNA. FT-IR spectra of the pineapple peel exhibit the absorption bands of chromophoric group characteristics of phenols, alcohols, amides, amino acids and proteins. The results suggest that this method showed a good potential with an ability to detect bioactive compounds early and accurately.

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