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Comparison of FTIR fingerprints in the fruits of *Pouteria campechiana* (Kunth) Baehni at different developmental stages

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ABSTRACT

Pouteria genus has wide ethnobotanical tradition as food, remedies or wood to general uses, most of the available scientific information is limited to few species with economical potential as source of eatable fruits, while several other species remain without information about their pharmacological and economical potential. Due to organoleptic and nutritional characteristics the fruit is consumed in its fresh ripe state. The edible flesh has been assessed in relation to antioxidant activity. Different phenolic acids, flavonols, and carotenoids have been identified and based on this the inclusion of such fruit in diet has been recommended. In this juncture present analysis is attempted to reveal FTIR spectra of the fruits at different developmental stages. Pouteria campechiana fruits at six different stages of development were collected (4WAP, 8WAP, 12WAP, 16WAP, 20WAP and 24WAP) and were subjected to FTIR spectral analysis. The resultant spectral peaks were analysed for the occurrence of characteristic functional groups that might represent the chemistry of various compounds present in the fruit pulp. Almost all stages of fruits were characterized by more or less similar peaks with different heights. Some of the peaks were shared and others unique were interpreted. The fourth stage (16 WAP) shows peaks at 2300 and 2330 cm^{-1} which specifically corresponds to C=O stretch of CO_2 absorption and it is at this stage the ripening process begins. The presence and absence of specific spectral peaks in different fruit stages can be correlated to the maturity stage and further to the specific metabolic shifts that are taking place in the plant in accordance with external and internal rhythms.

***Key words*:** *Pouteria, Functional groups, Fourier Transform Infrared Spectra*

INTRODUCTION

Objective- The aim of the present study was to analyze and compare the functional groups present in the fruits of *Pouteria campechiana* at different stages of development and maturation.

Plants are the potential source of medicine such as microbicidal, antioxidants, antiinflamatory and others[1]. World health organization (WHO) estimated that about 50% of the country's population exploits plants for their bioactive compounds especially in folk medicine. Such medicinal herbs possess an array of compounds like phenolics, alkaloids, saponins, terpenoids, and certain endogenous metabolites[2].

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The phenolicss are reported in many plants and they have many biological activities especially as therapeutic drugs to cure many ailment[3].

Chemotaxonomy has a potential role in the field of biology especially in plant systematics. Fourier Transform Infrared (FTIR) Spectroscopy is a rapid, noninvasive, high-resolution analytical tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint". This technology allows detecting the whole range of infrared spectrum in measurements of biological specimen[4]. The "fingerprints" are made up of the vibrational features of all the cell components, i.e., DNA, RNA, proteins, and membrane and cell-wall components. In plant classification, Kim *et al.*[5] have proposed this approach as robust in chemotaxonomic classification of flowering plants, and previously this method was used to identify the species in *Hypericum* L. and *Triadenum* Raf.[6]. The FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract. Ahamad .I *et al.*[7] detected major groups of compounds as the most active fraction of four plants extracts by infrared spectroscopy. Ramamoorthi and Kennan[8] screened the bioactive group of chemicals in the dry leaf powder of *Calotropis gigantea* by FTIR analysis.

Pouteria campechiana is an ever green tree native of South Mexico and Central America belongs to Sapotaceae[9]. The common name is canistel. In India it is known as egg fruit, usually grown in home gardens having 6 to 8 m in height. These plants show morphoforms which shows variation in leaf and fruit morphology. Fully ripened fruit is round, yellowish orange in colour represents the edible part. Indians do not consume or process the fruit due to the ignorance related its nutritional aspects. Several researchers have reported economic values to various parts of this plant[10]. The present study is the first attempt to trace out the phytochemical profile of the fruits at different stages of maturity or ripening stages through FTIR finger prints.

MATERIALS AND METHODS

Fruits of *Pouteria campechiana* at 6 different stages of maturity (4WAP, 8WAP, 12WAP, 16WAP, 20WAP and 24WAP) were collected. The finely chopped fruit tissues were shade dried to a crispy stage and then ground to a fine powder using a mortar and pestle. Samples were then subjected to FTIR spectral analysis using spectrophotometer. The characteristic peaks were analysed and compared.

Fourier Transform Infrared Spectrophotometer (FTIR)

FTIR is the 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 stages of fruit materials were used for FTIR analysis. 10 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 FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

RESULTS AND DISCUSSION

The FTIR spectrum of fruit samples are given in Fig 1 to 6. The data on the peak values and the probable functional groups (obtained by FTIR analysis) present in the samples are given in table 1. When the plant samples were passed into the FTIR, the functional groups of the components was separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of alcohol, phenol, alkanes, aldehyde, aromatic compound, secondary alcohol, aromatic amines and halogen compound.

The characteristic peaks in the range 1730-1734 cm^{-1} representing C=O stretch of aldehydes; 2900-2976 cm^{-1}, representing the C-H stretch of alkanes; 3300-3400 cm^{-1} corresponding to the N-H stretch of primary amines and 3500- 3550-3600 cm^{-1} representing the –OH stretch of alcohols and phenols are displayed by all fruits at all stages of maturity

(Fig. 1-6). Peaks in the range 730- 736 cm^{-1} are not shown by the 5^{th} and 6^{th} stages and these spectral peaks correspond to the C-H bend of alkynes. Further, peaks in the range 1026, 1029 and 1031 cm^{-1} representing the C-H stretch of aliphatic amines are found in these two last stages. The fruit at first stage

of development (4WAP) characteristically has spectral peaks at 626.87 corresponding to bromo-compounds and 900.76 representing aromatics. The third stage fruit (12WAP) do not display the peak at 580- 582 cm-1 which is shown by all other stages. Typically, spectral range of 500-600 characterise alkyl halides, specifically C-Br halides. Similarily, the fruits at the 1st and 6th stages lack the C≡N stretch of nitriles indicated by the spectral peaks 1247, 1249 and 1251. The first three stages additionally have peaks at 3045 and 3074 characterising C-H stretch of alkenes. Ironically, these three stages lack peaks at 3601, 3606 and 3614 corresponding to alcohols and phenols while the last three stages i.e., 4th, 5th and 6th stages possess them. The first stage distinctly lacks the N-H bend of primary amines as evidenced by the absence of peaks at 1614 and 1627. Strikingly, it has been observed that the 4th stage fruit has the unique peaks in the range 2331 and 2360 cm^{-1} (Fig. 4) specific for the C=O stretch of CO_2[11,12]. It has been observed that the ripening process initiates at this stage and proceeds further. The elevated CO_2 production suggests the climacteric feature of the fruits and that further accounts for the peaks which subsequently disappears at the mature stages of the fruits. In addition, many functional groups can be identified by their characteristics vibration frequencies making the IR spectrum simplest and most reliable method of assigning a compound to its class.

The spectral analysis reveals that *Pouteria* fruits at different stages of development display several functional groups like aldehydes, alkanes, primary amines, alcohols and phenols. The occurrence of specific peaks in some stages can be attributed to the biochemical as well as physiological need that influence the fruit development and maturation.

The Fourier Transform Infrared (FTIR) spectroscopy has proven valuable tool for characterization plant products. Moreover, FTIR spectroscopy is an established time saving method to characterize and identify functional group[13]. The spectra showed bands between 1630-1600 cm^{-1} in all stages of fruits explains the presence of pectins, an important class of heterogenous polysaccharides in the cell wall[14]. The phytochemical constituents of *Morinda citrifolia* fruit extracts were analyzed for the functional groups by FTIR spectroscopy confirmed the presence of polymeric hydroxyl group (3456cm^{-1}), cycloalkanes (2926 $cm^{-1}$and 2855cm^{-1}), ketones (1742cm^{-1}), aldehyde (1634cm^{-1}), sulphonic acid esters (1192cm^{-1}), alkenes (1113cm^{-1}), phenol (984cm^{-1}), aromatic compound (657cm^{-1}), and halogens (618cm^{-1})[15]. The polymeric hydroxyl groups, aromatic compounds, phenols, aldo-ketogroup peak further confirms the presence of polyphenols of which flavonoids, coumarins, anthroquinones and phenolic compounds are commonly present in the plants attribute to their antioxidant power. The FT-IR interferogram of date dietary fibres of three date palm cultivars at three maturity phases was carried out by Haider *et al.*[16] which suggest the occurrence of specified spectral peaks characteristic of chemical constituents. In Durian fruits, immature, mature, ripe and over ripe samples were analyzed for polyphenols which showed different bands[17].

Kareru *et al.*[18] carried the spectral analysis for saponins in the crude dry powder of *Albizia anthelmintica*, *Senna singueana*, *Maytenus senegalensis*, *Senna didymomotrya*, *Terminalia brownii*, and *Prunus africana*. The phytochemicals were likely to be bidesmosidic, oleanane-type triterpenoids, while those detected in *Entada leptostachya* and *Rapanea rhododendroides* might be monodesmosidic saponins. FTIR and EDS spectral analysis of *Eclipta alba* and *Eclipta prostrate* showed the presence of characteristic functional groups of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, nitrates, chlorates, and carbohydrate that are responsible for various medicinal properties of both herbal plants. The *Eclipta alba* contains a higher percentage of useful elements like Na, Mg, K, Ca, Cu, Zn, and Fe than *Eclipta prostrata*. In addition, *Eclipta prostrata* contains more of the toxic element Cd than *Eclipta alba*[19]. The FTIR analysis of methanolic and aqueous leaf extracts of *Bauhinia racemosa* revealed the presence of protein, oil, fats, phenolic compounds, flavonoids, saponins, tannins and carbohydrate as major functional groups[20]. Ragavendran *et al.*[21] screened the functional groups of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons, halogens that are responsible for various medicinal properties of *Aerva lanata*. Starlin *et al.* [22] analyzed the ethanolic extracts of *Ichnocarpus frutescens*, by FTIR, revealed functional group components of amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic hydrocarbons and halogens. Pednekar and Raman[23]

analyzed the methanolic leaf extract of *Ampelocissus latifolia* by FTIR and reported that the transition metal carbonyl compounds and aliphatic fluoro compounds were only present in the extract.

Torres-Rodríguez[24] analyzed soluble phenols and antioxidant activity in mamey sapote (*Pouteria sapota*) fruits in postharvest stage. Ashok kumar and Ramaswamy[25] evaluated the phytochemicals by FTIR spectroscopic analysis of leaf extracts of selected Indian medicinal plants and their therapeutic potentials. Packialakshmi and Naziya[26] employed Fourier transform infrared spectroscopy analysis of various solvent extracts of *Caralluma fimbriyata* and their medicinal significance. Maobe and Nyarango[27] validated *Warburgia ugandensis* the medicinal herb used for the treatment of diabetes, malaria and pneumonia in Kenya by FTIR spectra. Mariswamy *et al*.[28] applied FTIR Spectroscopic studies on *Aerva lanata* and their characteristic functional groups.

Table 1. FTIR spectral peaks characteristic of different stages of *Pouteria* fruit and the respective functional groups

Stages of Pouteria fruit	Range of IR spectral peaks (Cm^{-1})	Specified functional groups
Stages from 1-6	1730-1734	C=O stretch of aldehydes
	2900-2976	C-H stretch of alkanes
	3300-3400	N-H stretch of primary amines
	3500-3600	O-H stretch of alcohols and phenols
	1600-1630	Pectins
Stage 1-4	730-736	C-H bend of alkynes
Stages 5 & 6	1026,1029,1031	C-H stretch of aliphatic amines
Stages 1,2,4,5 & 6	58-582	Alkyl halides (C-Br halides)
Stages 2,3,4 & 5	1247,1249,1051	C≡N stretch of nitriles
Stages 1,2 & 3	3045,3074	C-H stretch of alkenes
Stages 4,5 & 6	3601,3606, 3614	Alcohols and phenols
Stage 1 only	626.87	bromocompound
	900.76	aromatics
Stage 4 only	2331, 2360	C=O stretch of CO_2

Fig. 1:

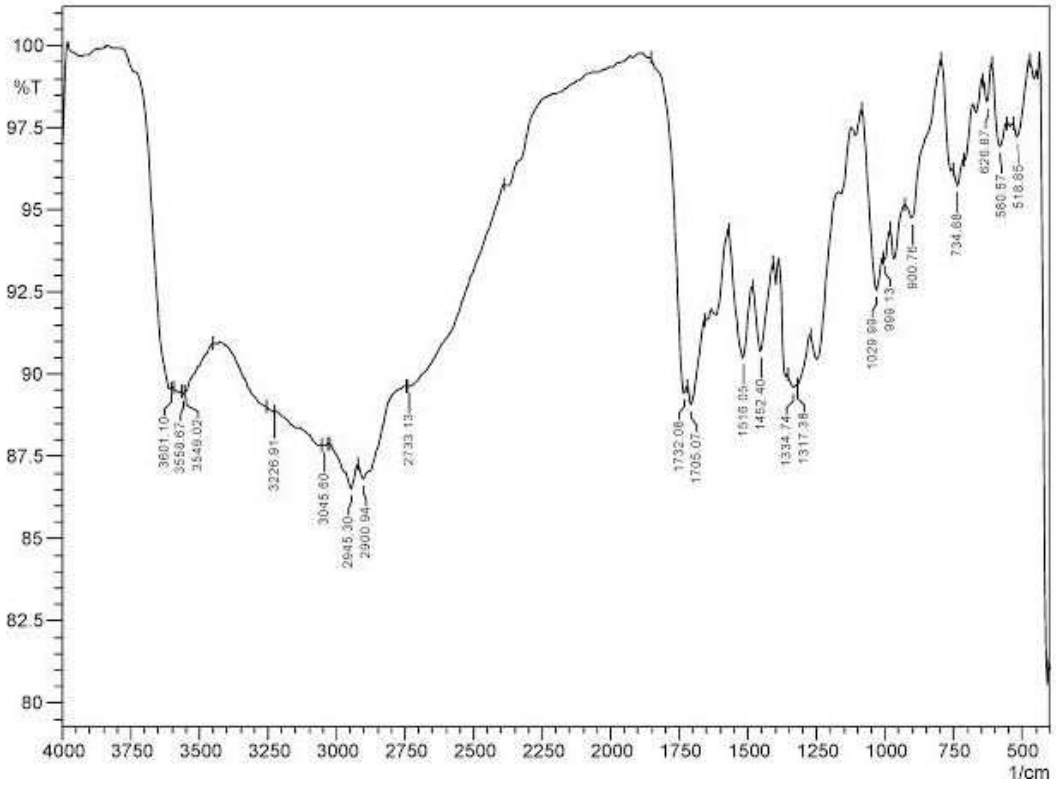


Fig. 2:

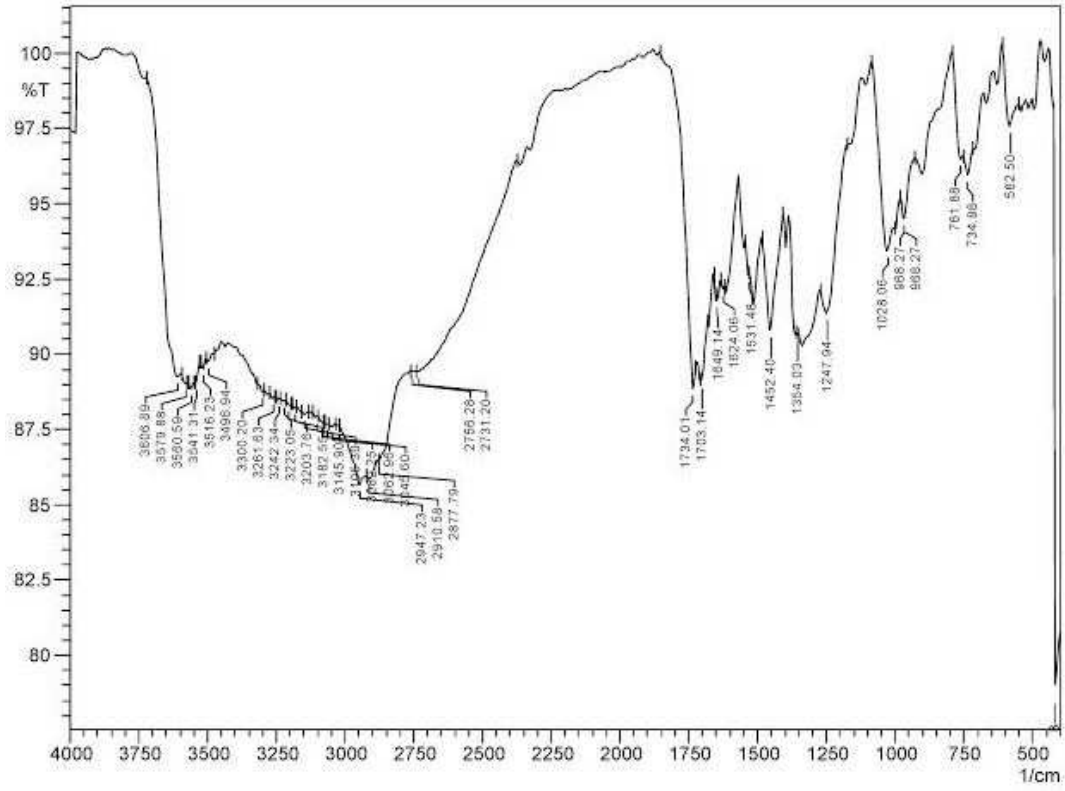
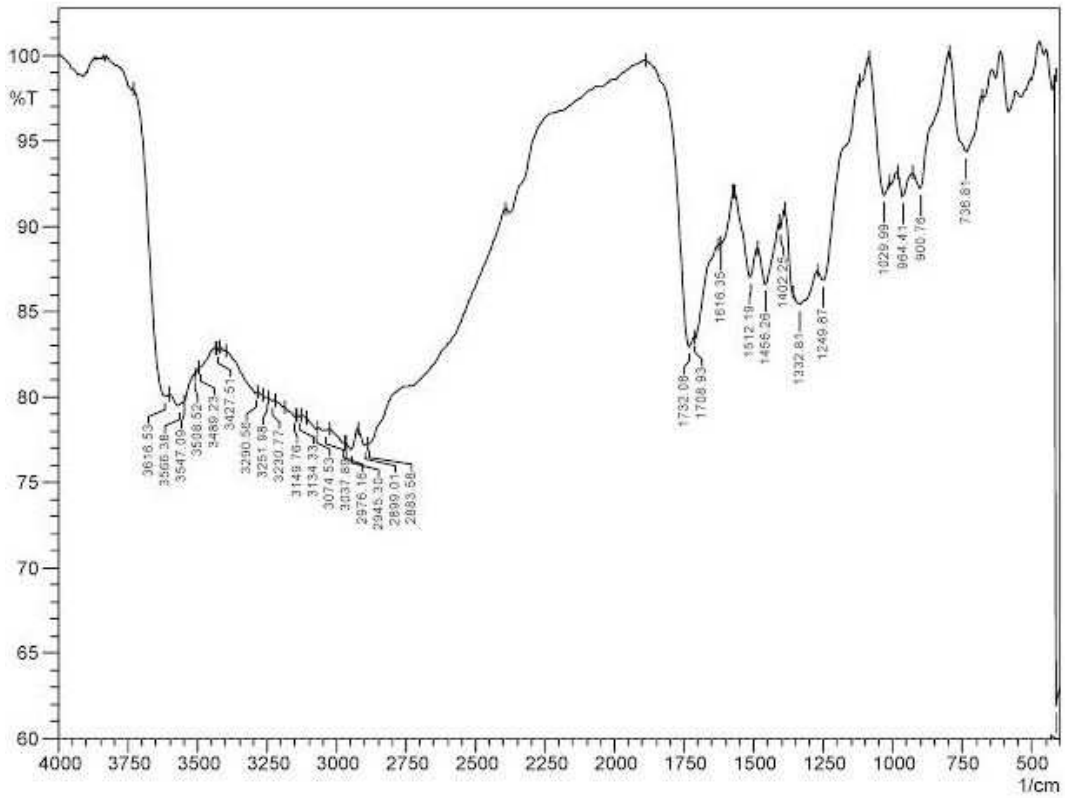

100
%T
97.5
95
92.5
90
87.5
85
82.5
80
4000 3750 3500 3250 3000 2750 2500 2250 2000 1750 1500 1250 1000 750 500
1/cm
3608.89
3579.88
3560.59
3516.23
3496.94
3300.20
3261.63
3242.34
3223.05
3203.76
3182.55
3145.90
2947.23
2910.58
2877.79
2756.28
2731.20
1734.01
1703.14
1649.14
1624.06
1531.48
1452.40
1384.03
1247.64
1028.06
966.27
908.27
761.88
734.98
582.50

Fig. 3: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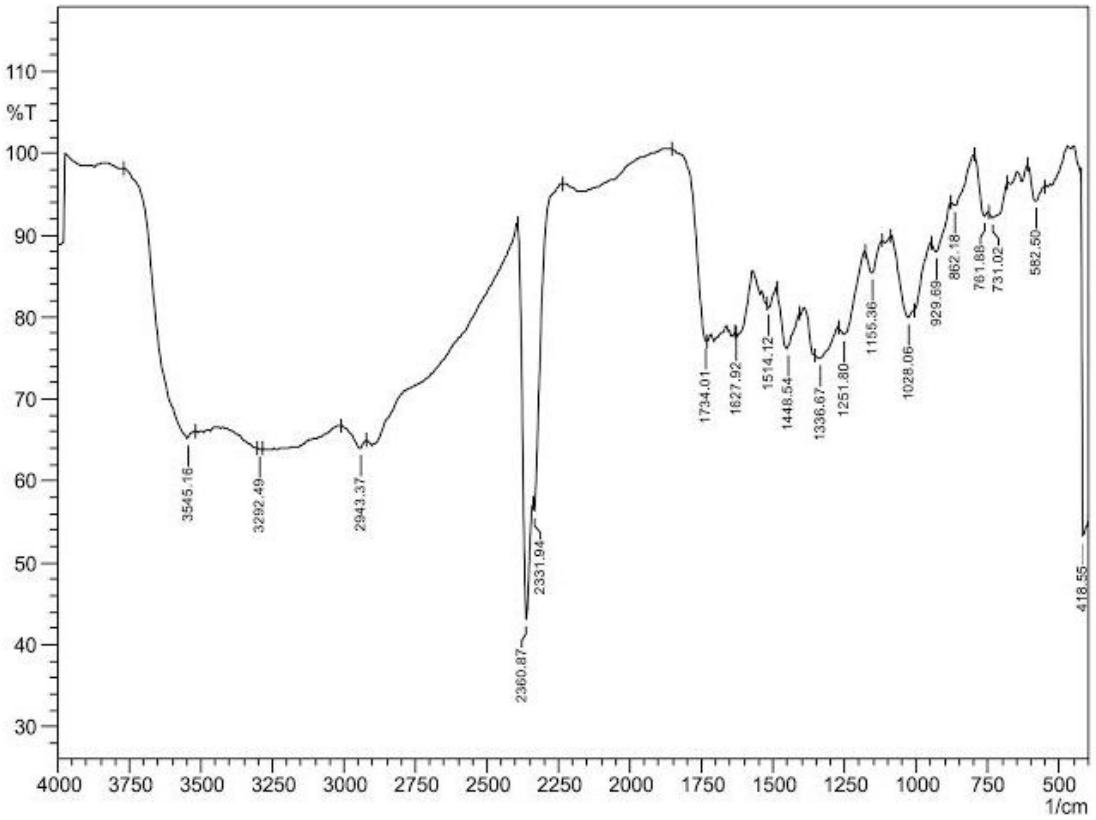
4000 3750 3500 3250 3000 2750 2500 2250 2000 1750 1500 1250 1000 750 500
1/cm
3616.53
3566.38
3547.09
3508.52
3488.23
3427.51
3251.98
3230.77
3149.76
3134.33
3074.53
2976.16
2945.35
2899.01
2883.58
1732.08
1708.93
1616.35
1512.19
1456.26
1402.25
1332.81
1249.87
1029.99
964.41
900.78
736.81

Fig. 4:


110
%T
100
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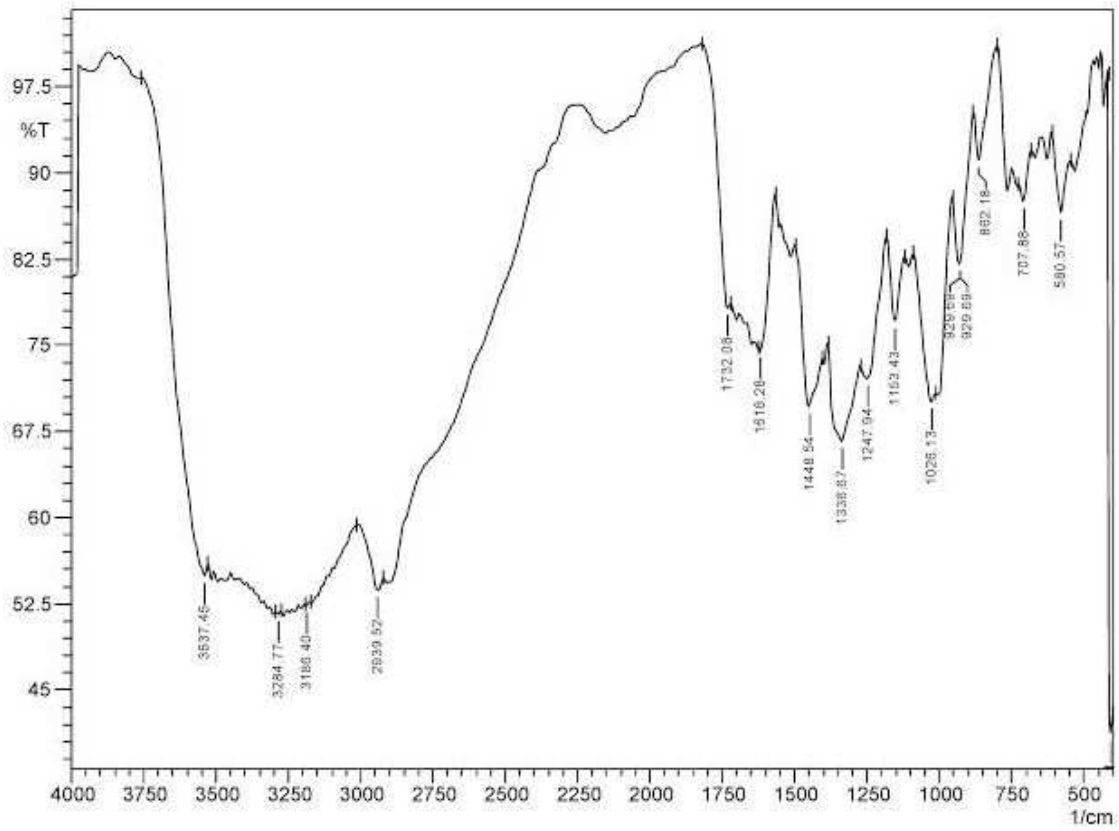
80
70
60
50
40
30
3545.16
3292.49
2943.37
2360.87
2331.94
1734.01
1627.92
1514.12
1448.54
1336.67
1251.80
1155.36
1028.06
929.69
862.18
781.88
731.02
582.50
418.55
4000 3750 3500 3250 3000 2750 2500 2250 2000 1750 1500 1250 1000 750 500
1/cm

Fig. 5:


97.5
%T
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67.5
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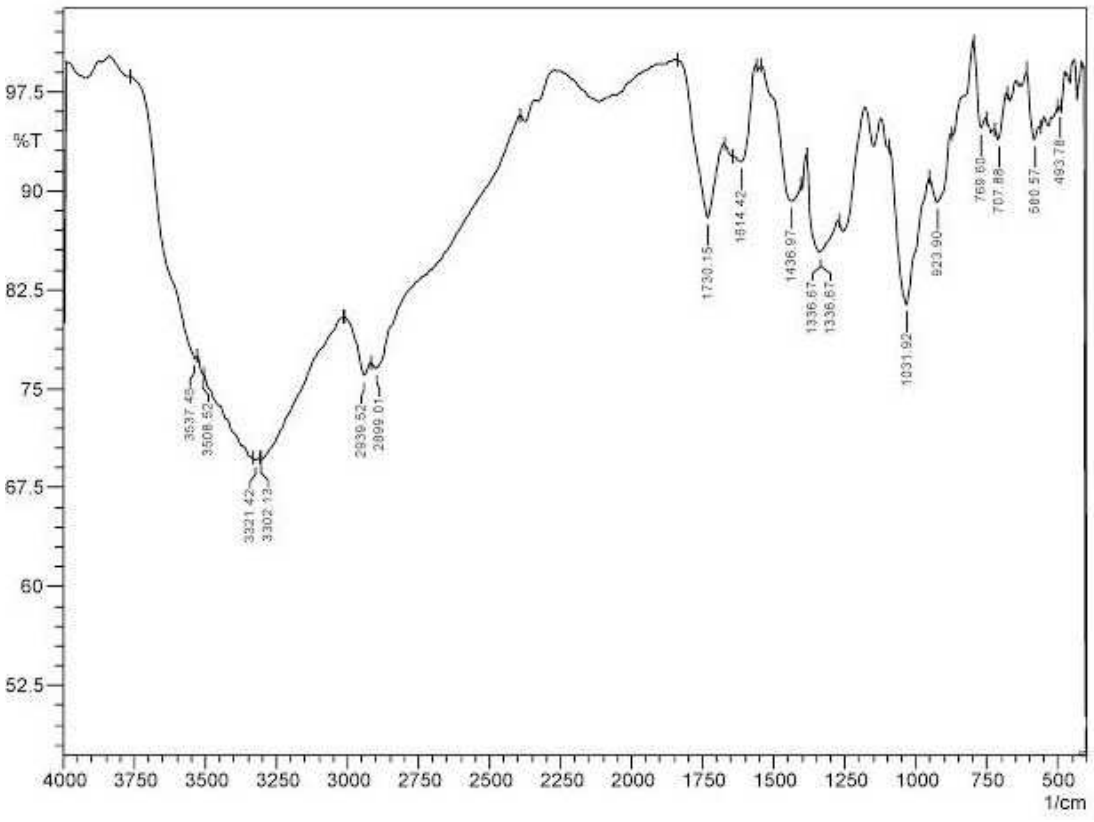
45
3537.45
3284.77
3186.40
2939.52
1732.08
1610.26
1448.54
1338.87
1247.94
1162.43
1026.13
929.69
862.18
707.88
580.57
4000 3750 3500 3250 3000 2750 2500 2250 2000 1750 1500 1250 1000 750 500
1/cm

Fig. 6:



CONCLUSION

The present study provides an insight into the functional groups available in the tissues of *Pouteria* fruits at different developmental maturity stages. It can provide valid data regarding the chemical profile or its change along with the initiation of ripening. Although there are many listed correlation between chemical structure and IR absorption peaks, the actual interpretation of a complex spectrum is difficult and the operation requires much experience. Additional analyses by employing tools like HPLC, HPTLC and NMR are warranted to decipher the exact nature of these compounds which can pave the way for their biochemical correlation.

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