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Research Article

EVALUATION OF ANTIOXIDANT POTENTIAL AND FOURIER TRANSFORM INFRARED SPECTROSCOPY ANALYSIS OF *ANANUS COMOSUS* (L.) MERRILL PEEL

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ABSTRACT
Ananas comosus (L.) Merrill is belonging to the family Bromeliaceae which act as an anti-inflammatory, antitumor effect. The present study is aimed to appraise the antioxidant potential and Fourier transform infrared spectroscopy (FTIR) analysis in *Ananus comosus* peel. The enzymatic and non enzymatic antioxidants profiles were analyzed in fresh peel of pineapple with the standard protocols and the FTIR was applied and Infrared spectrum in mid infrared region 4000–400 cm–1 was used for discriminating and to identify various functional groups present in the ethanolic extract of *Ananus comosus*. The fresh peel shows the significant amount of antioxidants and the FTIR analysis also found the presence of amino acids, alkenes, nitrates, nitrites, ethers, esters, aldehydes, alkynes, aromatic compounds, organic halogen compounds, carbohydrates, sulphur derivatives and polysaccharides. In conclusion, the results presented in the peel of *Ananus comosus* have strong antioxidant content which may be act as good pharmacotherapeutic agents in future.
Keywords: *Ananas comosus*, Bromeliaceae, Fourier transform infrared spectroscopy, antioxidants, pharmacotherapeutic agents

INTRODUCTION

Free radicals are fundamental to many biochemical processes and represent an essential part of aerobic life and metabolism. ROS mediated oxidative damage to macromolecules namely lipids, proteins and DNA have been implicated in the pathogenecity of major diseases such as cancer, rheumatoid arthritis, post ischemic reperfusion injury, degeneration process of aging, myocardial infarction, cardiovascular disease etc[1]. Antioxidants have been reported to prevent oxidative damage caused by free radicals by interfering with the oxidative process through radicals past few years for their antioxidant properties[2].

In recent years, secondary plant metabolites previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents. Phytochemicals like carotenoids, tocopherols, ascorbates and phenols present in the plants are strong antioxidants and have an important role in the health care system. There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. Screening active compounds from plants has lead to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer and Alzheimer's disease[3]. Fourier transform infrared spectroscopy (FTIR) is one of the most widely used methods to identify the chemical constituents and elucidate the compounds structures, and has been used as a requisite method to identify medicines in pharmacopoeia of many countries. Owing to the fingerprint characters and extensive applicability to the samples, FTIR has played an important role in pharmaceutical analysis in recent years[4].

Ananas comosus (L.) Merrill is belonging to the family Bromeliaceae and it is an important tropical and subtropical plant widely cultivated in the tropical areas of the world. Pineapple has also been known for a number of beneficial biological activities such as antioxidative, anti-browning, anti-inflammatory and anti-platelet activities. The enzyme complex of *A. comosus* called bromelain is known for its clinical applications particularly modulation of tumor growth, blood coagulation and anti-inflammatory effect[5]. Hence the present study is aimed to appraise the antioxidant potential and FTIR analysis in ethanolic extract of *Ananus comosus* peel.

MATERIALS AND METHODS

Sample collection and extraction

Fresh pineapple peel was collected from Coimbatore, Tamil Nadu, India. The plant was authenticated by Dr. P. Sathyanarayana, Botanical survey of India, TNAU Campus, Coimbatore and the voucher specimen No.BSI/SRC/5/23/2011/Tech-515. The sample was washed under running tap water. The samples were prepared by grinding one gram each of the pineapple peel in 2 ml of 50% ethanol, separately, in a pre-chilled mortar and pestle and the extracts were centrifuged at 10,000 g at 4ºC for 10 minutes. The supernatants thus obtained were used within four hours for various enzymatic and non-enzymatic antioxidants assays.

Assay of Superoxide Dismutase (SOD)

The assay of superoxide dismutase was done according to the method of[6]. In this method, 1.4ml aliquots of the reaction mixture (comprising 1.11 ml of 50 mM phosphate buffer of pH 7.4, 0.075 ml of 20 mM L-Methionine, 0.04ml of 1% (v/v) Triton X-100, 0.075 ml of 10 mM Hydroxylamine hydrochloride and 0.1ml of 50 mM EDTA) was added to 100µl of the sample extract and incubated at 30ºC for 5 minutes. 80 µl of 50 µM riboflavin was then added and the tubes were exposed for 10 min to 200 W-philips fluorescent lamps. After the exposure time, 1ml of Greiss reagent (mixture of equal volume of 1% sulphanilamide in 5% phosphoric acid) was added and the absorbance of the color formed was measured at 543 nm. One unit of enzyme activity was measured as the amount of SOD capable of inhibiting 50% of nitrite formation under assay conditions.

Assay of Catalase (CAT)

Catalase activity was assayed by the method of[7]. The enzyme extract (0.5 ml) was added to the reaction mixture containing 1ml of 0.01 M phosphate buffer (pH 7.0), 0.5 ml of 0.2 M H_2O_2, 0.4 ml H_2O and incubated for different time period. The reaction was terminated by the addition of 2 ml of acid reagent (dichromate/acetic acid mixture) which was prepared by mixing 5% potassium dichromate with glacial acetic acid

(1:3 by volume). To the control, the enzyme was added after the addition of acid reagent. All the tubes were heated for 10 minutes and the absorbance was read at 610 nm. Catalase activity was expressed in terms of µmoles of H_2O_2 consumed/min/mg protein.

Assay of Glutathione peroxidase (GPX)

Glutathione peroxidase was assayed according to the method of[8] with some modifications. The reaction mixture consisting of 0.4 ml of 0.4 M sodium phosphate buffer (pH 7.0), 0.1 ml of 10mM sodium azide, 0.2 ml of 4 mM reduced glutathione, 0.1 ml of 2.5 mM H_2O_2, 0.2 ml of water and 0.5 ml of enzyme was incubated at 0, 30, 60, 90 seconds respectively. The reaction was terminated with 0.5 ml of 10% TCA and after centrifugation, 2 ml of the supernatant was added to 3 ml of phosphate buffer and 1ml of DTNB reagent (0.04% DTNB in 1% sodium citrate). The color developed was read at 412 nm and the enzyme activity is expressed in terms of µg of glutathione utilized/min/mg protein.

Assay of Glutathione S-transferase (GST)

Glutathione s-transferase activity using chlorodinitrobenzene or dichloronitrobenzene as substrates was assayed spectrophotometrically essentially as described by[9]. The cuvettes (final volume of 3.0 ml) contained 0.1 M phosphate buffer (pH 6.5), 1 mM GSH and 1 mM of chlorodinitrobenzene and 20 μl of appropriately diluted enzyme from the different sources. Change in absorbance at 340 nm was followed against a blank containing all reactants excepting enzyme protein, Specific activity was expressed as µmol conjugate formed/min/mg protein.

Assay of Peroxidase

The assay was carried out by the method of[10]. The reaction mixture consisted of 3ml of buffered pyrogallol [0.05 M pyrogallol in 0.1 M phosphate buffer (pH 7.0)] and 0.5 ml of 1% H_2O_2. To this added 0.1 ml enzyme extract and O.D. change was measured at 430 nm for every 30 seconds for 2 minutes. The peroxidase activity was calculated using an extinction coefficient of oxidized pyrogallol (4.5 litres/mol).

Assay of Ascorbate Oxidase

Assay of ascorbate oxidase activity was carried out according to the procedure of[11]. The sample was homogenized [1: 5 (w/v)] with phosphate buffer (0.1 M/ pH 6.5) and centrifuged at 3000 g for 15 min at 50°C. The supernatant obtained was used as enzyme source. To 3.0 ml of the substrate solution (8.8 mg ascorbic acid in 300 ml phosphate buffer, pH 5.6), 0.1 ml of the enzyme extract was added and the absorbance change at 265 nm was measured for every 30 seconds for a period of 5 minutes. One enzyme unit is equivalent to 0.01 O.D change per min.

Assay of Polyphenol Oxidase (PPO)

Assay of Polyphenol oxidase activity was carried out according to the procedure of[12]. To 2.0ml of enzyme extract add 3.0ml of distilled water and mixed well. To this added 1.0ml of cathecol solution (0.4mg/ml) and the reactants were quickly mixed. The enzyme activity was measured as change in absorbance/min at 490nm.

Estimation of reduced glutathione (TRG)

The amount of reduced glutathione in the samples was estimated by the method of[13]. 1ml of the sample extracts were treated with 4.0 ml of metaphosphoric acid precipitating solution (1.67 g of glacial metaphosphoric acid, 0.2 g EDTA and 30 g NaCl dissolved in 100ml water). After centrifugation, 2.0 ml of the protein-free supernatant was mixed with 0.2 ml of 0.4 M Na_2HPO_4 and 1.0 ml of DTNB reagent (40 mg DTNB in 100 ml of aqueous 1% tri sodium citrate). Absorbance was read at 412 nm within 2 minutes. GSH concentration was expressed as nmol/mg protein.

Estimation of Vitamin C

The determination of ascorbic acid was carried out by the procedure given by[14]. The assay mixture for vitamin C consisted of 0.1 ml of brominated sample extract, 2.9 ml of distilled water, 1 ml of 2% DNPH reagent and 1-2 drops of thiourea. After incubation at 37ºC for 3 h, the orange-red osazone crystals formed were dissolved by the addition of 7 ml of 80% sulphuric acid and absorbance was read at 540 nm after 30 minutes. Vitamin C concentration was expressed in terms of mg/g tissue.

Assay of Malondialdehyde (MDA)

Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS), using malondialdehyde (MDA) as standard by the method of[15]. 1.0 ml of the sample extract was added with 2.0 ml of the TCA-TBA-HCl reagent (15% (w/v) TCA, 0.375% (w/v) TBA and 0.25N HCl). The contents were boiled for 15 minutes, cooled and centrifuged at 10,000 g to remove the precipitate. The absorbance was read at 535 nm and the malondialdehyde concentration of the sample was calculated using extinction coefficient of 1.56 x 105 M^{-1} Cm^{-1}.

Statistical analysis

The results obtained were expressed as mean±SD. The Statistical comparison among the groups were performed a statistical package program (SPSS 10.0).

FTIR spectrum analysis

100g of dried powder was extracted in 500ml of ethanol for 24hrs in occasional shaker at room temperature. The supernatant was collected and evaporated to make the final volume one-fifth of the original volume. It was stored at 4°C in airtight container. The dried sample was used for the analysis of FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan). The ethanolic extract was ground into fine powder by using agate mortar and the FT-IR spectrometer in the region 4000-400cm-1 by employing standard KBr pellet technique. The detector used in the system was Standard DLATGS with a mirror speed of 2.8 mm/sec[16].

RESULTS AND DISCUSSION

The body posses defense mechanisms against free radical-induced oxidative stress, which involve preventative mechanisms, repair mechanisms, physical defenses and antioxidant defenses. Enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) etc., while non-enzymatic antioxidants are ascorbic acid (vitamin C), α- tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids etc[17]. All these act by one or more of the mechanisms like reducing activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen. It is possible to reduce the risks of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants[18].

SOD plays an important role in protecting cells against ROS. It catalyzes the reaction of superoxide radical converting into water and oxygen. Catalase is an antioxidant enzyme widely distributed in all plant and animal tissues. The enzyme is known to protect the system from highly reactive hydroxyl radical through hydrogen peroxide decomposition[19]. Figure 1 shows the levels of SOD and Catalase in *Ananus comosus* peel. The levels were found to be 51.57±0.94 units/mg protein and 62.46±0.66 µmole of H_2O_2 consumed/min/mg proteins. The levels were expressed in mean±SD.

Figure 2 represents the levels of glutathione peroxidase and gluthathione s-transferase in *Ananus comosus* peel. The levels of these antioxidants were found to be 191.60±1.2 µg of glutathione oxidized/min/mg protein and 482.33±0.99 µmoles of CDNB – GSH conjugate formed/min/mg protein. GPX is a seleno-enzyme two third of which (in liver) is present in the cytosol and one third in the mitochondria. It catalyzes the reactions of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide[20]. GST catalyzes the conjugation of thiol group of glutathione to electrophilic substrates, and thereby detoxifies endogenous compounds such as peroxidized lipids[21].

Reduced glutathione is one of the most abundant non-enzymatic antioxidant bio-molecules present in tissues. Its functions are removal of reactive oxygen species and provision of a substrate for GPx and glutathione S-transferase (GST)[22]. Ascorbic acid is most powerful antioxidant under physiological conditions. It exists mostly in the reduced form. It can directly scavenge superoxide, hydroxyl radicals and single oxygen[23]. The levels of total reduced glutathione and vitamin C of *Ananus comosus* were found to be 73.26±1.9 µg/mg protein and 472.15±5.5 µg/mg protein respectively (figure 3).

The levels of ascorbate oxidase and peroxidase in *Ananus comosus* peel were shown in figure 4. The amount of the above mentioned antioxidants were found to be 19.67±1.52 unit/g sample and 473.66±0.57 µmoles/g sample respectively. Ascorbate oxidase catalyzes the oxidation of ascorbic acid to dihydroascorbic acid. The definitive biological function of ascorbate oxidase is not clear, although it has been reported that the enzyme may participate in a redox system involving ascorbic acid. In plants, antioxidant enzymes namely catalase and peroxidase have been shown to increase when subjected to stress condition. Peroxidase reduces HO to water while oxidizing a variety of substrates. Thus, peroxidases are oxidoreductases which use H O as electron acceptor for catalyzing different oxidative reactions[24].

Polyphenol oxidases are copper proteins catalyze the aerobic oxidation of certain phenolic compounds to quinones. The activities of these enzymes are important with regard to defense mechanism against diseases. LPO refers to the oxidative degradation of lipids that impairs cell membrane functions resulting into cell damage and leading to several pathologies and cytotoxicity. Malondialdehyde (MDA), a reactive electrophile species which is one of the LPO end product analogies to advanced glycation end product is used as redox marker and measurement for elevated oxidative stress[25]. Figure 5 shows the levels of polyphenol oxidase and LPO in *Ananus comosus* were estimated in fresh peel. The content of the above mentioned enzymes were found to be 1.00±0.2 µmoles/g sample and 3.71±1.16 nM of MDA formed/min/mg protein respectively.

FTIR spectrum analysis

FTIR spectroscopy was useful for the compound identification and when run under IR region in the range of 400 - 4000 cm-1 there was a variation in the peaks in both the plant samples[26]. From table 1 and figure 5 shows the strong peak at 3379.29 cm–1 assigned to the O–H stretching vibration and the peak at 1639.49, 1705.07, 1726.29 cm–1 assigned to the C=O stretching vibration means that some carbonyl compounds existed in the ethanolic extract. So, depending on the fingerprint characters of the peaks positions, shapes and intensities, the fundamental components are well in the materials can be seen clearly[27]. The peaks at 1600–800 cm–1 attributed to C=O stretching (lipids) and 1600–720 cm–1 attributed to the region of Amide I band of tissue protein (highly sensitive to the conformational changes in the secondary structure; amide I band is due to in-plane stretching of the C=O bond, weakly coupled to stretching of the N–H bond. The more intense bands occurring at 594.08, 1529.55, 1639.49, 1406.11, 1253.73, 1253.73, 1394.53, 1529.55, 1253.73, 1394.53, 1253.73, 1639.49, 175.07, 1726.29, 1406.11, 1444.68, 2929.87 cm–1 corresponding to C-N, NH^{3+} Bending, CO^{2-} Stretching, N=O stretching, O-$N0_2$ stretching symm. and a symm, O-N=O bending & N-O stretching, C=O stretching, O–H/N–H, C–H, C-CHO, CH_3 stretching, C=C stretching skeletal vibrations respectively indicate the presence of amino acids, alkenes, nitrates, nitrites, ethers, esters, aldehydes, alkynes, aromatic compounds, organic halogen compounds and carbohydrates in plant material[4]. There is no absorbance in between the region 2220-2260 cm-1 indicates that no cyanide groups in our plant extract[28].

CONCLUSION

The present study concluded that the significant amount of antioxidant profile and presence of amino acids, alkenes, nitrates, nitrites, ethers, esters, aldehydes, alkynes, aromatic compounds, organic halogen compounds, carbohydrates, sulphur derivatives and polysaccharides indulged in *Ananus comosus* peel will aid in the interpretation of clinical research in biological models are recommended for chronic diseases.

ACKNOWLEDGEMENT

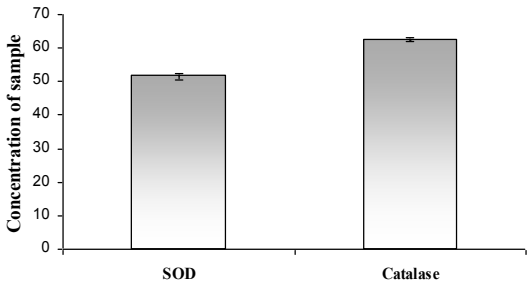
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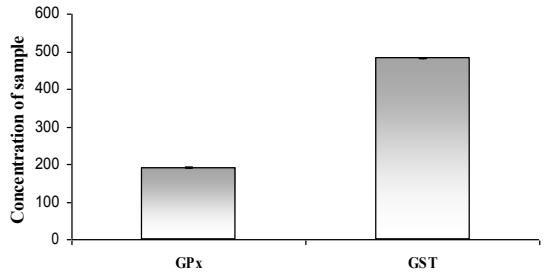
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Concentration of SOD and Catalase in *Ananus comosus*



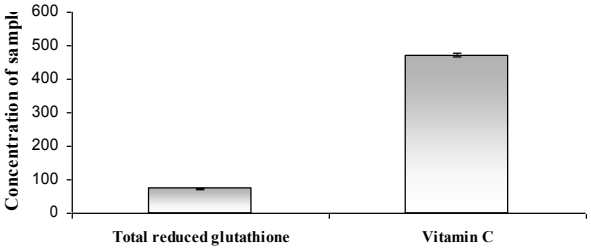
Values are expressed as mean±SD (n=3)
Units: SOD - Units/mg protein
Catalase - µmole of H_2O_2 consumed/min/mg protein
Figure 1: Effect of *Ananus comosus* on SOD and Catalase activity

Concentration of GPx and GST in *Ananus comosus*



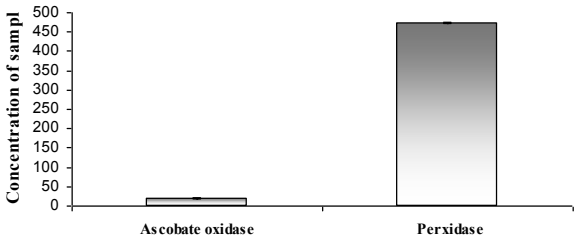
Values are expressed as mean±SD (n=3)
Units: GPx - µg of glutathione oxidized/min/mg protein
GST - µmoles of CDNB – GSH conjugate formed/min/mg protein.
Figure 2: Effect of *Ananus comosus* on GPx and GST activity

Concentration of Total reduced glutathione and Vitamin C in *Ananus comosus*



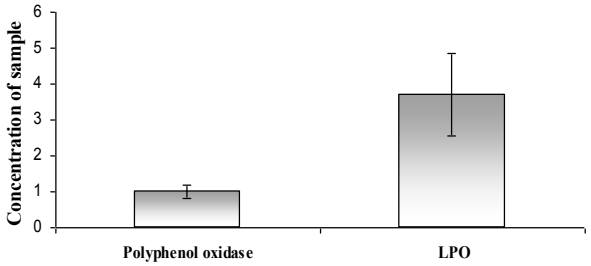
Values are expressed as mean±SD (n=3)
Units: Total reduced glutathione - µg/mg protein
Vitamin C - µg/mg protein
Figure 3: Effect of *Ananus comosus* on Total reduced glutathione and Vitamin C activity

Concentration of Ascorbate oxidase and Peroxidase in *Ananus comosus*

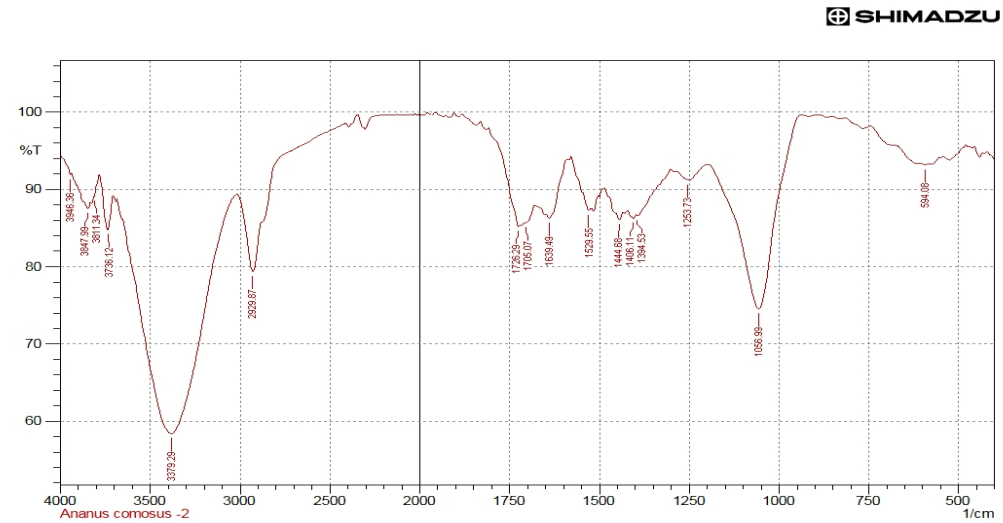


Values are expressed as mean±SD (n=3)
Units: Ascorbate oxidase – Unit/g tissue
Peroxidase - µmoles/g tissue
Figure 4: Effect of *Ananus comosus* on Ascorbate oxidase and Peroxidase activity

Concentration of Polyphenol oxidase and LPO in *Ananus comosus*



Values are expressed as mean±SD (n=3)
Units: Polyphenol Oxidase – µmoles/g tissue
LPO – nM of MDA formed/min/mg protein
Figure 5: Effect of *Ananus comosus* on Polyphenol oxidase and LPO activity



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	594.08	93.177	0.389	605.65	555.5	1.482	0.084
2	1056.99	74.54	22.219	1197.79	939.33	16.152	12.088
3	1253.73	91.24	1.417	1282.66	1199.72	2.984	0.297
4	1394.53	86.625	0.17	1396.46	1301.95	4.516	0.055
5	1406.11	86.247	0.664	1419.61	1398.39	1.334	0.046
6	1444.68	86.088	1.563	1485.19	1435.04	2.881	0.229
7	1529.55	87.278	0.816	1544.98	1523.76	1.189	0.059
8	1639.49	86.259	1.437	1647.21	1589.34	2.764	0.236
9	1705.07	85.653	0.207	1707	1680	1.681	0.039
10	1726.29	85.222	0.999	1809.23	1720.5	3.215	0.059
11	2929.87	79.389	11.346	3016.67	2414.88	20.489	5.44
12	3379.29	58.398	29.171	3653.18	3018.6	92.193	56.272
13	3736.12	84.786	5.416	3782.41	3709.11	4.258	1.086
14	3811.34	89.112	0.193	3813.27	3784.34	1.235	0.008
15	3847.99	87.507	0.824	3867.28	3834.49	1.841	0.074
16	3946.36	91.896	0.425	4000.36	3940.57	1.814	0.015

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Ananus comosus -2

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Figure 6: Fourier transform infrared spectroscopy analysis of *Ananus comosus* peel

Table 1: ASSIGNMENT OF IR ABSORPTION BANDS IN THE SPECTRA OF THE MEDICINAL PLANTS

FUNCTIONAL GROUPS	FREQUENCY RANGE (CM^{-1})	INTENSITY
AMINOACIDS C-N STRETCHING (ALIPHATIC & AROMATIC NITRO GROUP)	594.08	(M)
NH^{3+} BENDING (NH^{3+} ASYM & SYM BENDING)	1529.55, 1639.49	(M), (W)
CO^{2-} STRETCHING (CO_2 SYM. STRETCHING, NH^{3+} IN-PLANE BENDING)	1406.11, 1253.73	(W), (W)
NITRO COMPOUNDS ($N0_2$ ASYM. STRETCHING)	1529.55	(S)
NITROSO COMPOUNDS (N=O STRETCHING)	1253.73, 1394.53, 1529.55	(S), (S), (S)
NITRATE (O-$N0_2$ STRETCHING SYMM.) & O-$N0_2$ STRETCHING ASYMM.	1253.73, 1394.53	(S), (S)
NITRITE (O-N=O BENDING & N-O STRETCHING)	594.08, 1639.49	-
NITRAMINE (NO_2 SYM. STRETCHING AND NO_2 ASYM. STRETCHING)	1253.73, 1639.49	(S), (S)

NITROSAMINE (N-N STRETCHING & N=O STRETCHING)	1056.99, 1444.68	(S), (M)
HYDROXYL COMPOUNDS		
POLYMERIC (OH STRETCHING-BONDED)	3379.29	(S BROAD)
ASSOCIATED (INTRAMOLECULAR)	2929.67	(W), BROAD
(CONJUGATE CHELATION)	1394.53, 1406.11, 1444.68, 1529.55	(V) BROAD
(OH IN-PLANE BENDING)	1056.99, 1406.11, 1253.73,	(S), (S), (S), (S),
C-O STRETCHING AND O-H IN-PLANE BENDING	1394.53,	(S)
VIBRATIONS (COUPLED)	1406.11	(S)
ETHERS		
ARALKYL; ASYMMETRIC	1253.73	(S)
DIARYL, ANTISYMMETRIC	1253.73	(M)
ESTERS		
C=O STRETCHING	1639.49, 175.07, 1726.29	(S)
C-O-C STRETCHING	1253.73	(S)
-	1253.73	(S)
CARBOXYLIC ACID		
(C=O STRETCHING)	1705.07	(S)
(C-O STRETCHING AND O-H IN PLANE BENDING (COUPLED))	1394.53	(M)
CARBOXYLATES		
COO^- SYMMETRIC STRETCHING	1394.53, 1406.11	(S)
COO^- ASYMMETRIC STRETCHING	1639.49	(S)
CARBONYL COMPOUNDS		
C=O STRETCHING	1639.49, 1705.07, 1726.29	(S)
C-CO-C (SKELETAL)-CH_2 BENDING	1406.11	(S)
HALOGEN COMPOUNDS		
FLURO COMPOUNDS (C-F STRETCHING)	1056.99	(VS)
CHLORO COMPOUNDS (C-CL STRETCHING)	594.08	(S)
BROMO COMPOUNDS (C-BR STRETCHING)	594.08	(S)
IODO COMPOUNDS (C-I STRETCHING)	594.08	(S)
ALDEHYDES		
C=O STRETCHING	1705.07, 1726.29	(S)
C-CHO SKELETAL	1406.11, 1444.68, 1529.55	(V)
ALKANES		
C-H STRETCHING-CH_3 ASYMMETRIC STRETCHING	2929.87	(M)
CH_3 STRETCHING	2929.87	(W)
CH_2 ASYMMETRIC STRETCHING		
CH_2 SYMMETRIC STRETCHING	2929.87	(S)
C-H DEFORMATION (BENDING)-SYMMETRIC BENDING	2929.87	(M)
CH_3 BENDING VIBRATIONS		
CH_3 BENDING (SYM)	1394.53	(M)
CH_3 BENDING		
CH_2 OUT OF PLANE BENDING	1444.68	(M)
CYCLOALKANES-C-H STRETCHING		(V)
SYMMETRIC CH STRETCHING	1444.68	
ALKENES		(M)
C=C STRETCHING VIBRATION	1253.73, 1406.11, 1444.68	
C=C STRETCHING	1253.73	(M)
ALKYNES		(S)
C=C STRETCHING	2929.87	
AROMATIC COMPOUNDS		-
C=C STRETCHING VIBRATION	2929.87	
DO	1639.49	(VS)
DO	1639.49	(W)
DO	2929.87	BROAD
DO	1444.68	BAND, (M)
		(W)
		(M)

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