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Research Article

PHYTOCHEMICAL INVESTIGATION, FT-IR PROFILING AND ANTIBACTERIAL ACTIVITY OF PAPAYA (VAR. RED LADY) PEEL- A RICH SOURCE OF BIOACTIVE CHEMICALS

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ABSTRACT
Fruit wastes are produced in huge quantities which constitute a source of pesterer in the environment due to their high biodegradability. To explore the potential of fruit waste as natural resources of bioactive compounds the present study investigate the qualitative and quantitative analysis of phytochemicals, FT- IR fingerprinting and antimicrobial activity of petroleum ether, ethyl acetate, ethanol and water extracts of papaya peel collected from variety red lady. The qualitative phytochemical investigation of papaya peel extracts showed the presence of phenols, flavonoids, alkaloids, tannins, saponins, terpenoids and steroids, whereas in the estimation, total amount of phenols was high when compared to flavonoids. The FT-IR spectroscopic investigation showed the presence of characteristic peak values with different useful mixtures of functional groups such as hydroxy group, aliphatic, carbonyl, alcohols, phenols, alkanes, ketones, carboxylic acids, amine and aromatics. Among the four extracts evaluated, ethyl acetate fraction showed more numbers of functional groups. The ethyl acetate and ethanol extract displayed increased antibacterial activity in all tested bacterial strains and found as better solvents for the isolation of the bioactive compounds from papaya peel. Highest zone of inhibition (20mm) was showed by Gram negative bacterium *Proteus mirabilis* against synthetic antibiotic tetracycline (24mm).

Keywords: Papaya peel, Phytochemicals, Phenols, FT-IR, Antibacterial activity.

1. INTRODUCTION
The papaya peel discarded by fruit industries as by-products are typically considered as waste. The best and profitable way of management of fruit wastes is extraction of bioactive phytochemicals and its utilization in various sectors like cosmetics, pharmaceuticals and food industries [1]. The bioactive phytochemicals content is much higher in fruit peels when compared to the edible part [2]. The phytochemicals in plants can create various positive physiological effects on human body. Phytochemical screening could detect numerous important compounds which may be used as the bases of modern drugs for curing various diseases [3].

Phenolic as well as flavonoid compound are widely distributed in plants which have been reported to exert multiple biological effects including antioxidant, antibacterial, free radical scavenging abilities, anti-inflammatory and anticarcinogenic [4]. Thus a natural product plays an important role in the field of new drugs research and development because of their low toxicity, easy availability and cost effectiveness. FTIR Spectroscopy has been recognized as a major authoritative technique used for identifying the types of chemical groups present in the compounds. The wavelength of light fascinated is a characteristic of the chemical bond which might be seen in the annotated spectrum [5].

Antimicrobial activity of a plant helps to defend themselves against various pathogenic microbes by inhibition or killing mechanisms. The use of compounds derived from plant for antimicrobial treatment become popular and the secretion of these compounds are varying from plant to plant [6]. The diversity of pathogenic microorganisms which cause life-threatening infections have steadily increased especially in developing countries and they are becoming important causes of mortality in immune compromised patients. The use of commercially available anti-microbial drugs to cure numerous

pathogenic diseases causes multiple drug resistance among human pathogenic microorganisms [7]. Thus, the plant materials such as fruit peels which are thrown in the environment as agrowaste could be a source of novel antimicrobial compounds to overcome these antibiotic resistant organisms. In this scenario, the present study focused the phytochemical investigation, FT-IR Profiling and anti-bacterial activity of petroleum ether, ethyl acetate, ethanol and water extract of papaya peel of red lady variety in order to discover and develop novel therapeutic agents with improved efficacy which are useful in pharmaceutical and food industries.

2. MATERIAL AND METHODS

2.1. Soxhlet hot continuous extraction

Peel of Red Lady variety of papaya fruit collected from Kerala Agriculture University, Vellayani, Thiruvanantha-puram, Kerala was the plant material. 200 g papaya peel were continuously extracted using 500 ml petroleum ether, ethyl acetate, ethanol and water solvents by the method of soxhlet hot continuous extraction for eight hours. Then filtered extracts were concentrated by rotary evaporator. The extracted sample kept at 4°C for further analysis.

2.2. Qualitative Phytochemical Screening

The petroleum ether, ethyl acetate, ethanol and water extract of papaya peel were screened for the presence of different phytochemicals.

2.3. Quantification of Phenols and Flavonoids

Total phenol content was estimated by taking an aliquot of the petroleum ether, ethyl acetate, ethanol and aqueous extracts of papaya peel 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% Na_2CO_3 was added to the mixture and kept in boiling water bath for 1 min. The white precipitate removed by centrifuging it for few minutes 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% Na_2CO_3. 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 gallic acid (GAE) by taking absorbance against concentration. The total phenol content was calculated from the standard graph.

The total flavonoid content of the petroleum ether, ethyl acetate, ethanol and aqueous extracts were determined by $AlCl_3$ method with slight modification. 100µl of the extract was mixed with 100µl of 20% $AlCl_3$ 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 $AlCl_3$ as blank. Standard curve was made using quercetin (50-250 µg/ml) in methanol under the same condition. Total flavonoids expressed as µg quercetin equivalent/mg of extract.

2.4. Fourier Transform Infrared (FT-IR) Spectrophotometer

Fourier Transform Infrared (FTIR) Spectrophotometer is perhaps the most powerful tool for identifying the types of 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 FT-IR 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}

2.5. Antibacterial activity

The antibacterial potentiality of red lady variety of papaya peel (1 mg/ml) of four solvent extracts were done against *Staphylococcus aureus* (ATCC 29213), *Bacillus cereus* (ATCC 14579), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 700603) and *Proteus mirabilis* (GC 10) using agar well diffusion method [8]. The bacterial strains were brought from National Collection of Industrial Microorganism, Pune. Each test bacteria (0.1%) was inoculated with dried Mueller-Hinton agar (Himedia, Mumbai, India) plates. Four wells each of 10 mm were created in each plate with the help of a sterile well borer. 100 µl of each papaya peel solvent extract were loaded in four wells. Positive control was tetracycline 0.1 mg/ml and negative control DMSO (100%). Zone of inhibition was measured after incubation period (24 h, 37°C). Each experiment was performed in triplicates and the average diameter of zone of inhibition was recorded in millimeters. Mean and standard deviation of inhibition zones diameter from triplicate experimental data were calculated.

3. RESULTS AND DISCUSSION

3.1. Qualitative and quantitative phytochemical analysis

The data shown in Table 1 revealed the qualitative analysis of phytochemicals in different extracts of red lady variety of papaya peel. The tests reveal the presence of various bioactive secondary metabolites which might be responsible for their biological potentialities. Table 2 showed the quantitative analysis of flavonoids and phenols in petroleum ether, ethyl acetate, ethanol and water extract of papaya peel. Among the four extracts ethyl acetate showed highest total phenol content (88 µg of GAE/mg of extract) and ethanol resulted highest total flavonoid content (71.50 µg of QE /mg of extract).

Alkaloids have cytotoxic activity and diuretic properties, which affects the central nervous system and reduces appetite [9]. Various studies confirmed that saponins possess a unique property of precipitating and coagulating red blood cells [10]. Tannins have amazing stringent properties. They are known to hasten the healing of wounds and inflamed mucous membranes. Many tannin containing drugs are used in the treatment of piles, inflammation, burns and as astringent [11]. Highest amount of phenols were present in ethanol and ethyl acetate extract and moderately in petroleum ether and aqueous extract of papaya peel. They reported to exhibit antimicrobial, anti-inflammatory, anti-viral, anticancer actions [12]. Flavonoids are strongly present in petroleum ether, ethanol and water extract of papaya peel, prevent oxidative cell damage by acting as a potent water-soluble antioxidant as well as free radical scavenger and have strong anticancer activity [13]. Terpenoids are known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyper-glycemic, antiinflammatory and immunomodulatory properties. They are known to have insecticidal properties, so they can be used as protective substances in storing agriculture products as well [14, 15]. Steroids are responsible for cholesterol-reducing properties and regulating the immune response [16].

Table 1: Qualitative phytochemical tests of extracts of papaya peel

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

strong positive +++; moderately positive ++; low positive +; negative test -

Table 2: Quantitative analysis of total phenol and flavonoids of extracts of papaya peel

Sl. No.	Sample	Amount of phenol in Gallic acid (GAE) equivalent µg/mg of extract	Amount of flavanoid in quercetin equivalent µg/ mg of extract
1	Petroleum ether	61.40	12.54
2	Ethyl Acetate	88	35.63
3	Ethanol	72.21	71.50
4	Water	21.43	19.38

3.2. Fourier Transform Infrared Spectroscopy (FT-IR) analysis

FT-IR gives a fingerprint of sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material.

The petroleum ether extract of red lady variery of papaya peel exhibited a characteristic absorption band at 2954.82, 2922.75 and 2854.07cm^{-1} indicating the C-H stretching vibration due to the presence of alkanes and aliphatic compounds and the absorption at 1745.74 and 1711.72 cm^{-1} represented carbonyl group, bands at 1460.83 and 1377.27 cm^{-1} for C-H bending, at 1164.12 and 722.48 cm^{-1} indicate C-F stretching vibration for fluro compound. The band at 499.82 cm^{-1} represents C-I bending (Fig. 1).

The absorption spectra of ethyl acetate extract of papaya peel exhibited a peak at 2983.96 cm^{-1} represented the presence of alkane (CH stretch) and carboxylic acid. The peak at 1736.42cm^{-1} showed the presence of ester (C-O stretch) and aldehyde (C-O stretch). The peak at

1446.82 cm^{-1} showed the presence of aromatic (C=C stretch), 1372.50 cm^{-1} represented the presence of nitro (NO) stretch. The peak at 1233.33, 1096.78 and 1042.96 cm^{-1} represent C-F stretching vibration for fluro compound. The infrared spectrum with a frequency at 938.04, 846.93, 786.03, 607.70, 534.11 and 455.41 cm^{-1} are of esters, amines, alkyl halide and alkene (Fig.2).

Ethanol extract of peel of red lady variety of papaya peel exhibited a characteristic band at 3300.44cm^{-1} indicate OH stretching vibration of alcohols and phenols, the peak at 2928.68cm^{-1} represented the presence of alkane (CH stretch) and carboxylic acid (O-H stretch). The peak at 1635.21 cm^{-1} correspond to stretching vibration of C=C group and the peak of 1408.12 and 1237.41 cm^{-1} represent the presence of amines (C-N stretch) and alkyl halide (C-F stretch), at 1054.25 cm^{-1} indicate C-O group and peak of 506.38, 470.79 and 459.33 cm^{-1} represent the monosubstituted groups (Fig. 3).

The water extract showed characteristic absorption bands at 3259.70 cm^{-1} which represented OH stretching vibration of alcohols and phenols. A notable peak at 1597.85cm^{-1} for aromatic compounds, peak at 1404.08 cm^{-1} represent C-H stretching and at 1041.42 shows C-O group and at 943.05, 505.61, 470.61 and 459.94 cm^{-1} were probably of esters, aromatic, nitro and amines (Fig. 4).

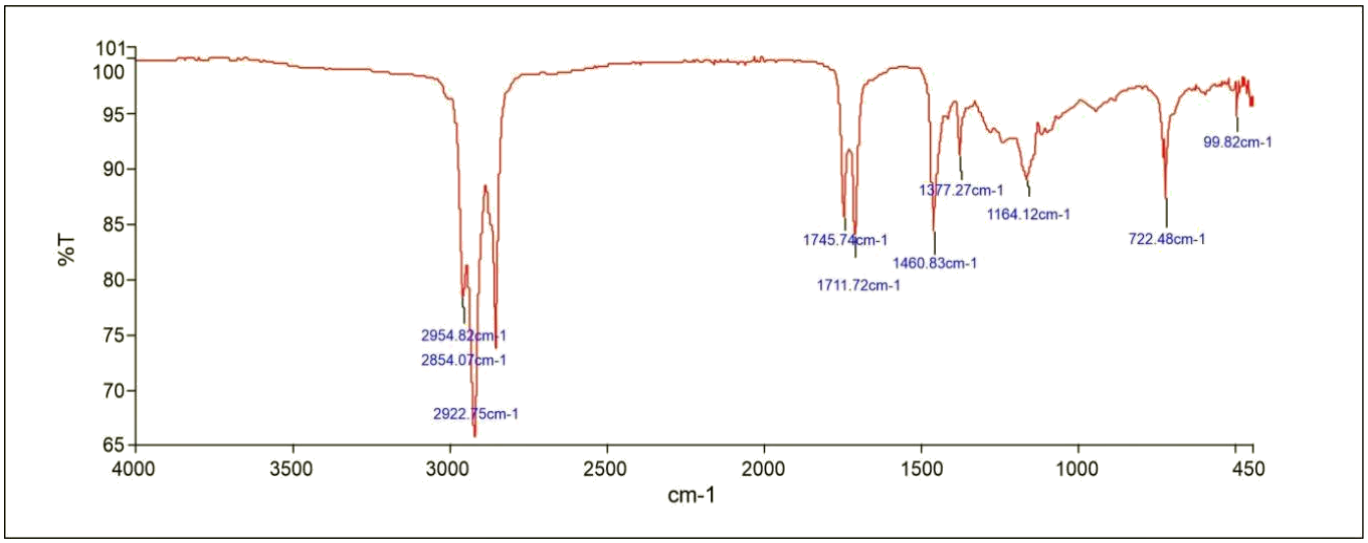


Fig. 1: FT- IR Spectral analysis of petroleum ether extract

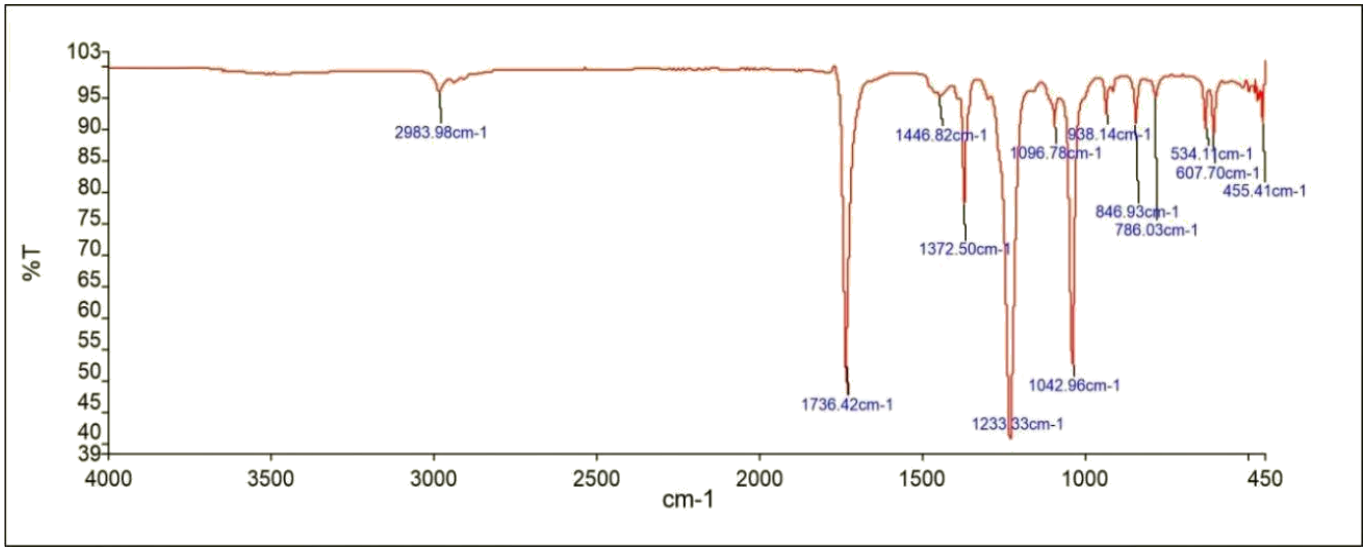


Fig. 2: FT- IR Spectral analysis of Ethyl acetate extract

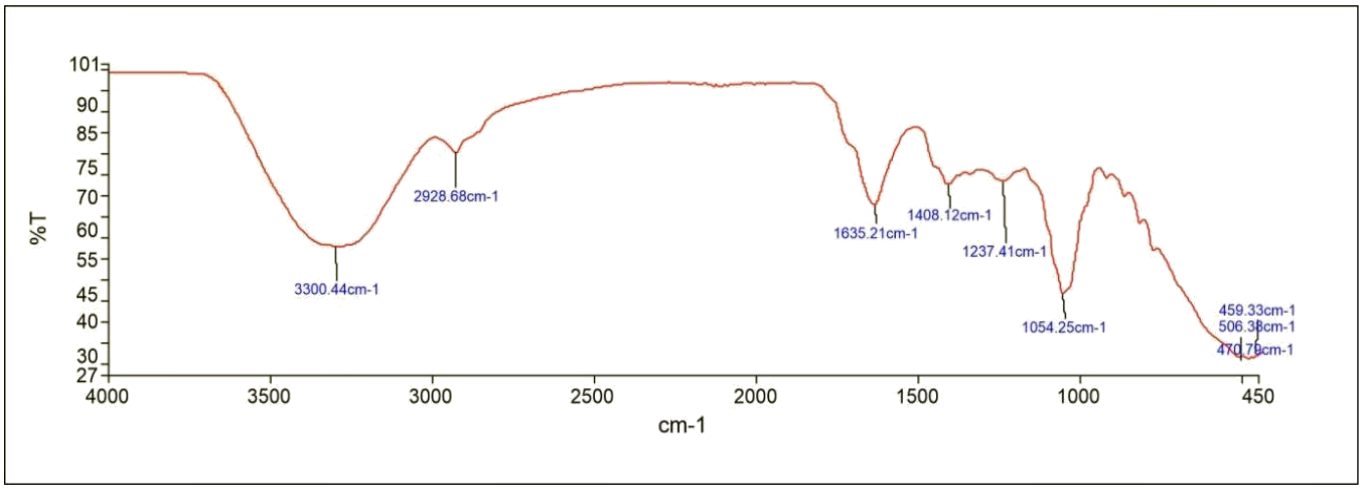


Fig. 3: FT- IR Spectral analysis of ethanol extract

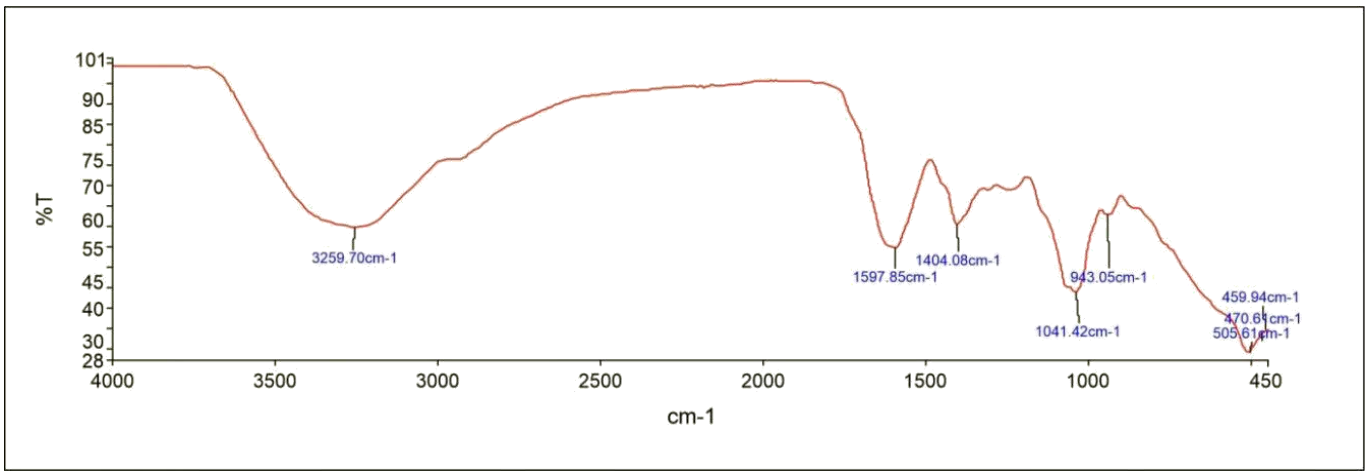


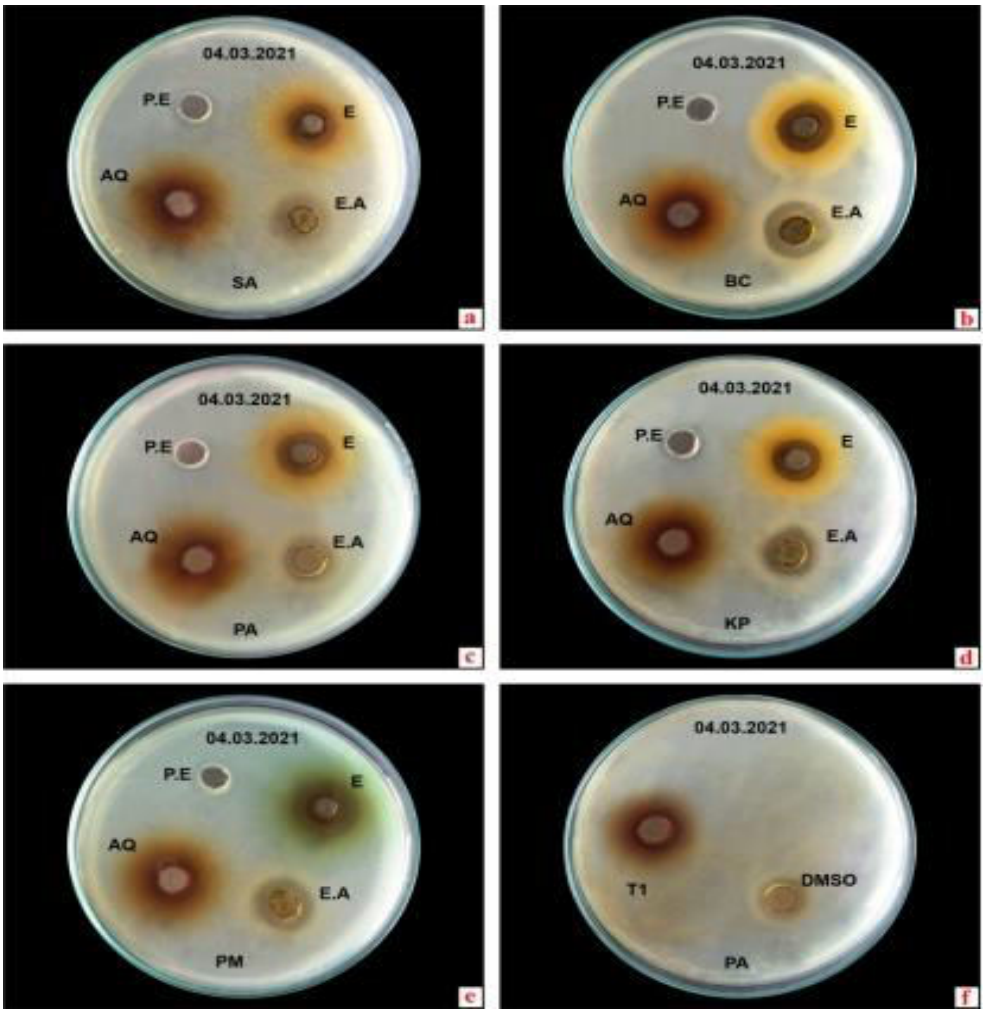
Fig. 4: FT- IR Spectral analysis of water extract

FT-IR analysis of papaya peel in petroleum ether, ethyl acetate, ethanol and water extract revealed numerous peaks which in turn confirms the presence of various functional groups like alcohols, phenols, alkanes, aliphatic compound, alkenes, carboxilic acid and fluro compounds. The highest number of peaks (13) were noticed in the ethyl acetate extracts followed by petroleum ether extracts (10), ethanol (9) and aqueous extract (8) respectively. Using FT-IR spectrum, we can confirm the functional constituent's presence in the extract. Muruganantham *et al*. (2009) [17] carried out the FT-IR spectral analysis of the medicinal plants, *Eclipta alba* and *Eclipta prostrata* and reported 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.

3.3. Antibacterial Activity

Because of the increasing antibiotic resistant microorganisms, it is very essential to find natural agents with antimicrobial potentials having new mechanisms of action. The antimicrobial potential of different solvent extracts in red lady variety of papaya peel was evaluated against *S. aureus, B. cereus, P. aeruginosa, K. pneumoniae* and *P. mirabilis*. The antibacterial activity of different extract was visualized by inhibition zone (Fig. 5 a-f). The diameters of inhibition zones at different concentrations of the extracts tested were shown in Table 3. Papaya peels in ethyl acetate and ethanol solvent extracts resulted the best antibacterial activities against tested pathogenic bacterial strains. This is probably because organic solvents have good efficiency to extract polyphenolic and other biologically active compounds from natural sources so that it can fight towards wide range of

bacteria. The low antimicrobial activity of papaya peel of water extracts may due to hydrophobicity [18, 19].
The Gram negative bacterium *Proteus mirabilis* showed highest inhibition zone (20mm) when compared with antibiotic tetracycline (24 mm) as the positive control. Variation in composition of biologically potential compounds in different extracts from papaya peel cause the observable differences in the levels of antibacterial activity (zone of inhibition) against all pathogenic bacteria tested. Microorganisms widely vary in their susceptibility to antimicrobial agents. The antibacterial activity exhibited by papaya peel might be due to individual or combined effects of the phytochemicals.



a. Staphylococcus aureus (SA), b. Bacillus cereus (BC), c. Pseudomonas aeruginosa (PA), d. Klebsiella pneumoniae (KP), e. Proteus mirabilis (PM), f. positive control (Tetracycline), negative control (DMSO) against Pseudomonas aeruginosa (PA)

Fig. 5 (a-f): Antibacterial potentiality of papaya peel of red lady variety in petroleum ether (P.E), ethyl acetate (E.A), ethanol (E) and water (AQ) extracts

Phytochemicals act as anti-bacterial, hormonal-stimulant component and effective binders so they can prevent pathogens attachment to walls of human cell [20]. The alkaloids compounds inhibit function of bacterial cell through various mechanisms by damaging its cell structure, protein which results in bacterial death [21]. The natural phenolic compounds have the ability to inhibit bacterial efflux pump and DNA gyrase enzyme [22]. The multiple target sites in bacterial cell wall are affected with compouds derived from phenolic acids, esters of weak acid, fatty acid and terpenes [23]. The lipophilic features of terpene contribute its antimicrobial mechanism, monoterpenes alters bacterial membrane structure which raises its fluidity, change the proteins topology which affect the bacterial respiration chain [24]. Gradisar *et al.* [25] studied the inhibitory effect in the functioning of DNA gyrase due to increased concentration of tannins. Kabuki *et al.* [26] reported that

the fruit peels may potentially possess antimicrobial property. Papaya fruit peel which is disposed as waste by the fruit industry, can be developed into a new functional drug that will shield humanity from the threat of antibiotic resistant microbes.

Table 3: Antibacterial potentiality of papaya peel extracts of red lady variety against pathogenic bacterial strains

Microorganisms	Red lady variety papaya peel extracts (concentration: 1mg/ml)	Inhibition zone (milimeter)
Staphylococcus aureus	Petroleum ether	12 ± 0.04
	Ethyl acetate	15 ± 0.07
	Ethanol	16 ± 0.08
	Water	13 ± 0.06
	Tetracycline	24 ± 0.15
	DMSO	0
Bacillus cereus	Petroleum ether	11 ± 0.02
	Ethyl acetate	17 ± 0.10
	Ethanol	16 ± 0.08
	Water	13 ± 0.06
	Tetracycline	24 ± 0.15
	DMSO	0
Pseudomonas aeruginosa	Petroleum ether	11 ± 0.02
	Ethyl acetate	15 ± 0.07
	Ethanol	16 ± 0.08
	Water	0
	Tetracycline	24 ± 0.15
	DMSO	0
Klebsiella pneumoniae	Petroleum ether	11 ± 0.02
	Ethyl acetate	16 ± 0.08
	Ethanol	15 ± 0.07
	Water	12 ± 0.04
	Tetracycline	24 ± 0.15
	DMSO	0
Proteus mirabilis	Petroleum ether	0
	Ethyl acetate	17 ± 0.10
	Ethanol	20 ± 0.12
	Water	13 ± 0.06
	Tetracycline	24 ± 0.15
	DMSO	0

Values of inhibition zone in mm were mean ± SD of three independent replications (0 = No zone of inhibition)

4. CONCLUSION

The present study concluded that the red lady variety of papaya peel extracts possess significant amount of phytochemicals, strong functional groups and marked antibacterial potentiality towards gram negative as well as gram positive bacteria, thus the thrown away waste can be used beneficially for the isolation of bioactive compounds which can be used in the development of phytomedicine.

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Conflict of interest

The authors declared there is no conflict of interest

6. REFERENCES

1. Qdais H, Abdulla F, Qrenawi L. *Jordan JMec Ind Eng*, 2010; **4**:69-74.
2. Joshi VK, Kumar A, Kumar V. *Intl J Food Ferment Technol*, 2012; **2**:123-136.
3. Sheikh N, Kumar Y, Misra AK, Pfoze L. *Journal of Medicinal Plants Studies*, 2013;**1**:62-69.

4. Cook NC, Samman S. *Journal of Nutritional Biochemistry*, 1996; **7**:66–76.
5. Sahayaraja PA, Gowri J, Dharmalingama V, Shobanaa R , Angelin Premab AA. *International Journal of Nano Corrosion Science and Engineering*, 2015; **2:**5.
6. Carvalho RS, Carollo CA, Magalhaes ,Boaretto AG, Ferraz AC, Limaa WG et al. *African Journal of Botany*, 2018; **114**:181-187.
7. Das K, Tiwari RKS, Shrivastava DK. *Journal of Medicinal Plants Research,* 2010; **4**:104-111.
8. Javed A, Venkatesha RT, Sagar S, Parameswaraiah MV, Ganguly D, Murugan S et al. *Am J PhytomedClinTher*, 2015;**3**:185-192.
9. Solanki R,Khanna M, Lal R. *Indian J Microbio*, 2008;**48**:410-431.
10. Okwu DE. *AgricEnviron*, 2004; **6**:30-37.
11. Kolodziej H, Kiderlen AF. *Phytochemistry*, 2005; **38:**2056-2071.
12. Aliyu AB, Musa AM, Sallau MS, Oyewale AO. *Trends in Applied Sci*ences Research, 2009; **4**:68-72.
13. Rio DA,Obdululio BG, Casfillo J, Marin FG, Ortuno A. *Agric Food Chem*, 1997 **45**:4505-4515.
14. Rabi T, Bishayee A. *Breast Cancer Res Treat*, 2009; **115**:223-239.
15. Sultana N, Ata A. *J Enzyme Inhib Med Chem*, 2008; **23**:739-756.
16. Shah BA, Qazi GN, Taneja SC. *Nat Prod Rep,* 2009; **26**:72-89.
17. Muruganantham S, Anbalagan G, Ramamurthy N. *Romanian journal Biophysics*, 2009; **19**:285–294.
18. Dean GB, Tricia LMD, Moriah MG, Ruben T. *Journal of Medicinal Chemistry*, 2014; **57**:10144-10161.
19. Iyer R, Ferrari A, Rijnbrand R, Erwin AL. *Antimicrobial Agents and Chemotherapy,* 2015; **59**:2388-2397.
20. Blessy BM, SureshKJ, Archana T. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2011; **4**:2.
21. Feng R, Qu J, Zhou W, Wei Q, Yin Z, Du Y. *International Journal of Clinical and Experimental Medicine,* 2006; **9**:22886-22892.
22. Farhadi T, Khameneh B, Iranshahi M, Iranshahy M. *Phytotherapy Research*, 2019; **33**:13-40.
23. Burt S. *International Journal of Food Microbiology*, 2004; **94**:223-253.
24. Paduch R, Kandefer-Szerszen M, Trytek M, Fiedurek J. *Archivum Immunologiaeet Therapiae Experimentalis*, 2007; **55**:315-327.
25. Gradisar H, Pristovsek P, Plaper A, Jerala R. *Journal of Medicinal Chemistry*, 2007; **50**:264-271.
26. Kabuki T, Nakajima H, Arai M, Ueda S, Kuwabara Y, Dosako S. *Alternate Medicine Review*, 2007; **1**:103.