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Evaluation & characterization of microbial pigments from the combination of pulp of *Carica papaya* and peels of *Citrus reticulata*

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Abstract
The present study was carried out for determination of the microbial pigments present in the combination of *Citrus reticulata* (peel) and *Carica papaya* are the medicinally important plants belonging to the Rutaceae & Caricaceae family respectively. In the present study, the extract of *Citrus reticulata* peel with *Carica papaya* pulp has been subjected to fermentation by using *Penicillium purpurogenum* and analyzed by analytical techniques. This analysis revealed that microbial extract of C*itrus reticulata* and *Carica papaya* contains 1,3-Butanediol, (S)-, 1,3 Propanediol, 2-Butanone, 3-Hydroxy, 2-Octanol, Benzofuran, 2,3-Dihydro, 2-Methoxy-4-Vinylphenol, 3-Methyl-1,6-Heptadien-3-Ol, Decanoic Acid, Ethyl Ester, 4-Hydroxy-3,5,5-Trimethyl-4-[(1e)-3-Oxo-1-Butenyl]-2-Cyclohexen-1-One, 8-Hydroxy-2h-Chromen-2-One, Hexadecanoic Acid, and Beta-Sitosterol.

Keywords: hexadecanoic acid, microbial pigments, beta.-sitosterol, fermentation, vinylphenol etc

Introduction
The orange peels are the byproducts amid the processing of foods grown from the ground demonstrate that they are great wellsprings of bioactive compounds [3]. Every year a lot of oranges waste squanders are framed, for example, peels [1] India creates around 25 Lakh huge amounts of Orange consistently. The principle orange creating conditions of India are Punjab, Madhya Pradesh, Andhra Pradesh, Maharashtra Rajasthan, Assam, and Karnataka [8]. During the generation of squeezed orange and other orange items, the orange peel gathers in the mass and will create ecologically issue. In this manner, it is fundamental to discover the applications for these peels. The orange peels are wealthy in supplements furthermore, contain numerous phytochemicals; subsequently they can be helpful in numerous medications and nourishment items [5]. This inquire about work is expected to separate orange peel by ordinary extraction procedure and fluid extraction and to assess the phytochemical substance of the orange peel.
From waste materials, there is dependably an expanded consideration in bringing helpful items and citrus squanders are no exemptions [2]. Natural contamination can likewise be diminished. The citrus peels are wealthy in supplements and contain numerous phytochemicals; they likewise can be effectively utilized as medications or as food supplements. [7, 4].

Materials
- The *Penicillium purpurogenum NCIM 713* - was purchased from NCI Pune, and the stock culture was maintained on a Potato Dextrose Agar (PDA) slants.
- The peels of *Citrus reticulata* and pulp of *Carica papaya* were collected in fresh container.
- PDB (2%), $MgSO_4$ (1%), $MnSO_4$(1%), K_2HPO_4 (1%) and KH_2PO_4 (1%) and Urea(0.5%) with pH 5.5

Methodology
Production of microbial pigments from peels of *Citrus reticulata* and pulp of *Carica papaya*
The peels were initially washed with tap water, followed by distilled water to remove soil and other contaminants. Then, equal amounts of pulp of papaya and peels of orange were weighed and ground into a paste and used as the carbon source.

Fermentation
10 ml of culture of the *Penicillium purpurogenum* was inoculated into a corresponding 250 mL Erlenmeyer flask containing 100 mL of production medium composed of PDB (2%), $MgSO_4$ (1%), $MnSO_4$(1%), K_2HPO_4 (1%) and KH_2PO_4 (1%) and Urea(0.5%) with pH 5.5. The inoculated flask was incubated on a rotary shaker (200 rpm) at 25ºC for 7-10 days.

Pigment extraction
After incubation, the broth obtained was taken and heated on a heating mantle at 70 degrees Celsius for 2 hours. After heating, the broth was filtered, separating the biomass and the filtrate. The pH of the filtrate was checked. The solution obtained was evaporated and concentrated at 70 degrees Celsius. The water molecules are slowly removed on evaporation leaving the solid concentrate. The concentrate was cooled immediately. The crude extract obtained was subjected to crystallization to form crystals of the pigment. The pigment obtained was purified and weighed.

UV-Visible Spectroscopy (UV-Vis)
The maximum absorbance of extracted and dried red pigment powder was determined by spectrophotometer (SPECORD 210-222K333 UV-Vis) at 500 nm wavelength [11].

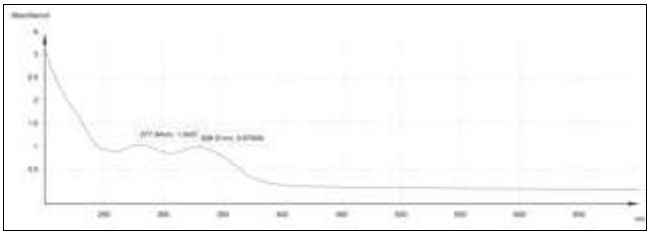

Fig 1: UV Spectra of Citrus *reticulata* and *Carica papaya*

Fourier Transform InfraRed (FT-IR) spectroscopy

The Fourier transform infrared (FT-IR) spectrum was recorded on a Bruker FT-IR spectrophotometer and the spectral range was 4000 – 500 cm-1 [9]. The dried powder of the red pigment was scanned by a Shimadzu FT-IR 8000 spectrophotometer in the 4000–400 cm−1 range using the KBr method at 27 °C.

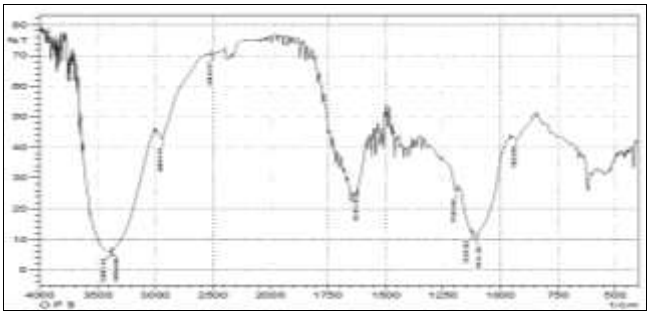

Fig 2: FTIR Spectra of Citrus *reticulata + Carica papaya*

Table 1: FTIR Peak value, range and bond type of Citrus *reticulata + Carica papaya*

Absorption	Absorption		Specific type of bond			
Peak value	range					
3383.14	3500-3300		1^O amines (doublet), 2^O amines N-H stretch			
3369.64	3500-3300		1^O amines (doublet), 2^O amines N-H stretch 2933.73		3000-2830	Alkanes C─H stretch
2501.67	2270–1930		Allenes, Ketenes, Isocyanates,			
				Isothiocyanates X=C=Y stretch		
1616.35	1640-1550		Amides, 1^O and 2^O amines N-H stretch			
1193.94	1200-1140		Sulfones, sulfonyl chlorides, sulfates,			
				sulfonamides S=O stretch		
1124.50	1300-1000		Alcohols, esters, ethers, -COOH,			
				Anhydrides C-O stretch		
1101.35	~1100			C─O bond (alcohols, secondary)		
929.69		1000-650		Alkenes C-H out of plane bend		
617.22		800-600		Chloride C-Cl stretch		

Nuclear Magnetic Resonance Spectroscopy

The purified pigment was dissolved with dimethylsulfoxide (DMSO d6) and the sample was injected into a nuclear magnetic resonance (NMR) spectrometer (Bruker 400 MHz) [10].

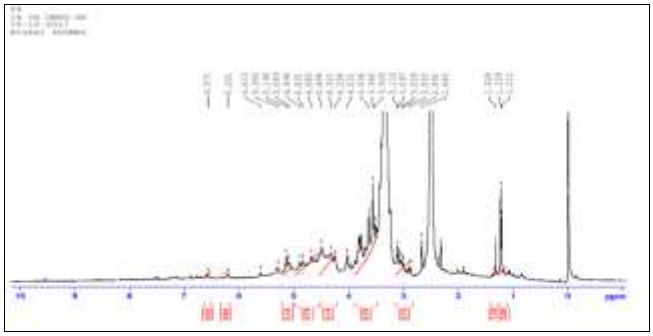

Fig 3: H-NMR-Spectra of *Citrus reticulata + Carica papaya*

Gas Chromatography-Mass Spectrometry

In the current study the microbial extract of fruit & pulp was screened for the presence of phytochemical compounds by qualitative test procedures followed by GC-MS analysis for detection of novel compounds. The mass spectra of compounds in the microbial extract was matched with that of NIST (National Institute of standards and Technology) and Wiley library.

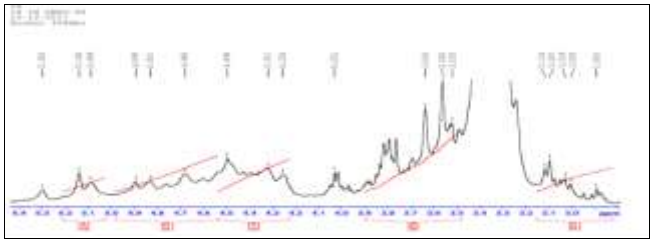

Fig 4: GC – MS Spectrum of *Citrus reticulata* & *Carica papaya*

Table 2: Major phytochemical compounds identified in microbial extract of *Citrus reticulata* & *Carica papaya*

S.no	RT(min)	Name of the compound	Molecular Formula	Mol Weight	Peak Area %	Pharmacological purpose
1	5.014	1,3-Butanediol, (S)-	$C_4H_{10}O_2$	90	6.42	Flavoring Agents, Food Additives: CARRIER_SOLVENT, Polyesters, polyurethanes, surface active agents; plasticizers, humectant, coupling agent, In cosmetic, pharmaceutical industry as glycerin substitute, 1,3-Butylene glycol efficient antimicrobial agent, inhibiting gram-neg & gram-pos microorganisms, molds & yeasts.
2	5.780	1,3 Propanediol	$C_3H_8O_2$	76	2.25	FDA Generally Recognized as Safe - GRAS Notices, Enzymes and Enzyme Stabilizers;Solvents, Intermediate, especially for polyesters, Heat transfer fluid, solvent, coolant
3	7.375	2-Butanone,	$C4H_8O_2$	88	5.32	Flavoring Agents, Aroma carrier; prepn of flavors & essences, Used for

		3-Hydroxy				flavoring margarine
4	8.150	2-Octanol	$C_8H_{18}O$	130	23.03	Flavoring Agents, CHEM INT FOR ALCOHOL-DERIVED SURFACTANTS
5	11.659	Benzofuran, 2,3-Dihydro	C_8H_8O	120	1.31	known as coumaran, a clear very slight yellow oily liquid
6	12.939	2-Methoxy-4-Vinylphenol	$C_9H_{10}O_2$	150	2.74	an aromatic substance used as a Flavouring agent, natural aroma of buckwheat.
7	16.135	Decanoic Acid, Ethyl Ester	$C_{12}H_{24}O_2$	200	2.14	Flavouring agent, found in alcoholic beverages
8	18.897	4-Hydroxy-3,5,5-Trimethyl-4-[(1e)-3-Oxo-1-Butenyl]-2-Cyclohexen-1-One {Dehydrovomifoliol }	$C_{13}H_{18}O_3$	222	0.38	found in cereals and cereal products
9	19.773	8-Hydroxy-2h-Chromen-2-One {8-Hydroxycoumarin}	$C_9H_6O_3$	162	0.20	Aromatic-hydroxylation-of-fused-benzene-ring-pattern2 occurs in humans.
10	20.449	Hexadecanoic Acid	$C_{16}H_{32}O_2$	256	0.53	Flavoring Agents, Enzyme Inhibitors, Surfactants, Manufacture of metallic palmitates, soaps, lubricating oils, waterproofing, food-grade additives
11	25.230	Beta.-Sitosterol	$C_{29}H_{50}O_8$	414	0.19	Hypolipidemic Agents, Sterols, Stigmasterols

Result

The conditions under which the pigment was produced were pH 5.5, temperature 37ºC and production time of 8 days. The highest production of peel and & pulp combination of pigments was 7% i.e., 70g/L yield and maximum UV absorbance at 277.94 nm (1.043). The produced pigment was characterized using different techniques like UV Visible Spectroscopy, FTIR and 1H NMR and GC-MS techniques.

Discussion

Now a day the study of the organic compounds from plants and their activity has increased. Gas Chromatography – Mass Spectrometry (GC - MS) is a valuable tool for reliable identification of bioactive compounds [6]. In the present study, 150 compounds have been identified from the microbial extract of combination of *Citrus reticulata* & *Carica papaya* by GC - MS analysis. The most abundant 11 components found in the combined extract were 1,3-Butanediol, (S)-, 1,3 Propanediol, 2-Butanone, 3-Hydroxy, 2-Octanol, Benzofuran, 2,3-Dihydro, 2-Methoxy-4-Vinylphenol, Decanoic Acid, Ethyl Ester, 4-Hydroxy-3,5,5-Trimethyl-4-[(1e)-3-Oxo-1-Butenyl]-2-Cyclohexen-1-One, 8-Hydroxy-2h-Chromen-2-One, Hexadecanoic Acid, And Beta.-Sitosterol.

The most significant outcome of this study was the production of yellow pigment from *Penicillium purpurogenum* under various nutritional conditions. It could be seen that *Penicillium purpurogenum* responded by producing high concentrations of pigment of orange peel with papaya pulp. The structural elucidation from GCMS shows that structure of the main pigment constituent in the pigment. To the best of our knowledge, this is the first study to report yellow pigment production by *Penicillium purpurogenum* from this fruit pulp and peel combination.

Acknowledgement

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Conflict of Interest

Authors declares that they had no conflict of interest. This article does not contain any studies with human participants or animals performed by any of the authors.

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