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UV-Vis Spectroscopy Analysis of Chlorophylls and Carotenoids in the leaves of *Carica Papaya L.* (Male and Female) by Using Various Extracting Solvents in kachchh and Saurashtra region

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Abstract: Present investigation is performed on the extraction of photosynthetic pigments like chlorophyll-a, chlorophyll-b and carotenoids in *Carica Papaya L.* (Male and Female). by using different solvent like methanol, ethanol, diethyle ether, acetone and DMSO. Different trend is observed in extraction rate for chlorophylls and carotenoids. Methanol was shown to have the highest extraction rate of carotenoids and chlorophylls (chlrophylls a and b) in the male *Carica Papaya L.* species from the Kachchh region. 95% ethanol demonstrates the maximum extraction of chlorophylls (Chlorophyll a and b) and carotenoids for female *Carica Papaya L.* species from the kachchh area. Dimethyl-sulphoxide (DMSO) exhibits the highest extraction of chlorophylls (Chlorophyll a and b) from the leaves of the male Carica Papaya L. Saurashtra species, while 95 %ethanol is the best solvent for carotenoids. The species chlorophylls (chlrophyll a and b) and carotenoids provide the greatest amount of extraction in diethyl-ether for female *Carica Papaya L.*from the Saurashtra region.

Key Word: Solvent extraction, chlorophylls, carotenoids, *Carica Papaya L.*, Kachchh, Saurashtra, UV-VIS Spectroscopy analysis

Abbreviations and notation: DMSO: Dimethyl-sulphoxide, DEE: Diethyl-ether

I. Introduction

More than just finding new medicines is studied in medicinal plant research.[1] Natural goods offer limitless potential for the novel medicine, whether they are used as pure components or standardized plant extracts.[2] This field has been growing and currently covers a wide range of topics, including power negotiation based on an understanding of medicinal plants.[3] Arthro glycosides, bitter substances, flavonoids, alkaloids, saponins, coumarins, phenol carboxylic acids, and terpenes are only a few of the various phytoconstitutents found in plants.[4] These phytoconstitutents endow plants with particular traits and abilities.[5]

Determining the diverse biological activities of plants would therefore benefit from the examination of their elements. For the qualitative and quantitative examination of pharmaceutical and biological materials, the spectroscopic approach has developed into a potent analytical tool.[6]There is a need for simple, affordable, and quick assays to find phytocomponents.[7] In this regard, both traditional procedures and spectroscopic (UV-Vis) methods can be applied. Ultraviolet-visible spectroscopy (UV-Vis) related to the quantitative method for calculating how much a chemical substance absorbs light is called spectroscopy (or spectrophotometry).[8] This is accomplished by comparing the amount of light that passes through a sample to the amount of light that goes through a reference sample or a blank.[9] The visible spectrum or its nearby wavelengths are used in UV-visible spectroscopy.[10] The absorption in the visible ranges is directly influenced by the colour of the substances involved. In various portions of the electromagnetic spectrum, molecules go through electronic transitions.[11]

Carica Papaya L. is an important plant species traditionally used in Indian system of medicine mainly for the treatment of various diseases such as asthma, arthritis, joints pain, ear ache, dog bite, chronic ulcer, fever, cough, cold, menstrual disorder, bone fracture, bronchitis, dysentery, dyspepsia, tumor, constipation, skin diseases, ascites and stomach distension.[12,13,14] The main objective of the present study is to identify the phytoconstituents like chlorophyll-a, chlorophyll-b and carotenoids of *Carica Papaya L.* leaves extract by UV-VIS spectrum.

II. Material And Methods

Collection of plant sample: The plant *Carica Papaya L.* was collected from the region of Kachchh and Saurashtra, Gujarat, India. The authenticity of the plant was confirmed in Botanical Survey of India. The whole plant was washed under running tap water, shade dried at room temperature and powdered. Preparation of plant extract for UV-VIS spectrum analysis the shade dried whole plant of Carica papaya L (at 25 °C) was powdered in a mechanical grinder. Analysed for the determination of chlorophylls (Ch-a and Ch-b) and carotenoids content.

Analytical procedure: Accurately weighted 0.5g of fresh plant leaves of *Carica Papaya L.* Sample was taken, and homogenized in tissue homogenizer with 10 ml of different extractant solvent. Homoginized sample mixture was centrifuge for 5000 rpm for 15min at 25ºC. The supernatant were separated and 1 ml of it is mixed with 4 ml of the respective solvent. The solution mixture was analysed for Chlorophyll-a, Chlorophyll-b and carotenoids content in UV spectroscopy (shimadzu). The equation used for the quantification of Chlorophyll-a, Chlorophyll-b, and carteniods by different extractant solvents are given in table 1; and spectral absorbance for Chlorophyll-a, Chlorophyll-b, and carteniods for various solvents are represented in table.

Quality control: Analytical reagents used during the extraction process were of AR grade (Merck). Milli Q water was used for preparation of intermediate solution and for dilution purpose (wherever needed). Quartz cuvette (1cm2) was used and corresponding solvent was taken as reference during spectrophotometric observation. Every procedure (for each plant sample and extracting solvent) was triplicated for maintaining the precision of analytical results.

Table-1 Equations to determine concentrations (µg/ml) of chlorophyll a (Ch-a), chlorophyll b (Ch-b) and total carotenoids (C x+c) by different extractant solvents in UV spectroscopy[15, 16, 17]

Solvents	Formula
95% Ethanol	Ch-a=13.36A664 – 5.19 A649 Ch-b=27.43A649 – 8.12 A664 C x+c=(1000A470 –2.13Ca- – 97.63Cb)/209
Methanol	Ch-a=16.72A665.2 – 9.16A652.4 Ch-b=34.09A652.4 – 15.28A665.2 C x+c=(1000A470 – 1.63Ca – 104.96Cb)/221
80% Acetone	Ch-a=12.25A663.2 – 2.79A646.8 Ch-b=21.5A646.8 – 5.1A663.2 C x+c=(1000A470 – 1.82Ca –85.02Cb)/198
Diethyl-ether (DEE)	Ch-a=10.05A660.6 – 0.97A642.2 Ch-b=16.36A642.2 – 2.43A660.6 C x+c=(1000A470 – 1.43Ca – 35.87Cb)/205
Dimethyl-sulphoxide (DMSO)	Ch-a=12.47A665.1 – 3.62A649.1 Ch-b=25.06A649.1 – 6.5A665.1 C x+c=(1000A480 – 1.29Ca-53.78Cb)/220

A = Absorbance, Ch-a = Chlorophyll a, Ch-b = Chlorophyll b, C x+c = Carotenoids

III. RESULTS AND DISCUSSION

The qualitative UV-VIS spectrum profile of solvent extract of *Carica Papaya L.* was selected at a wavelength from 190 to 800 nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks absorbance respectively.

The primary pigments that transform light energy into chemical energy are known as chlorophyll-a. By transmitting the light it absorbs to chlorophyll-a, chlorophyll-b, an auxiliary pigment, indirectly contributes to photosynthesis.[18]The chlorophyll molecule has an ionic and hydrophilic center made of Mg^{2+}, and a hydrophobic ring with a carbonyl group at its tail that is polar in nature.[19] Water-soluble chlorophyll-binding protein keeps it in place within the plant cell (WSCP). Only one functional group (i.e., -CHO) attached to the porphyrin ring distinguishes chlorophyll-b from chlorophyll-a, and because of this, chlorophyll-b is more soluble than chlorophyll-a in polar solvents.[17]

Structure: Chemdraw structure of Chlorophyll-a and Chlorophyll-b

Structure: Chemdraw structure of Carotenoids

Table-2 UV spectroscopic determination of absorbance for Chlorophyll a, Chlorophyll b and Carotenoids with various extracting solvents.

Extractant solvent	**Carica papaya male leaves kachchh**	**Carica papaya female leaves kachchh**	**Carica papaya male leaves Saurashtra**	**Carica papaya female leaves Saurashtra**
95 % Ethanol				
A664nm Ch-a	0.526	0.647	0.654	0.711
A649nm Ch-b	0.279	0.362	0.341	0.369
A470 nm C x+c	0.690	0.890	1.023	0.903
Methanol				
A665.2 nm Ch-a	0.652	0.299	0.387	0.169
A652.4nm Ch-b	0.417	0.192	0.223	0.108
A470 nm C x+c	1.023	0.434	0.447	0.225
80 % Acetone				
A663.2 nm Ch-a	0.584	0.531	0.452	0.763
A646.8 nm Ch-b	0.291	0.295	0.204	0.398
A470 nm C x+c	0.721	0.704	0.552	0.890

Diethyle ether				
A660.6 nm Ch-a	0.509	0.457	0.320	0.926
A642.2 nm Ch-b	0.176	0.209	0.132	0.488
A470 nm C x+c	0.468	0.534	0.384	1.909
Dimethyl sulfoxide				
A665.1 nm Ch-a	0.387	0.215	0.711	0.464
A649.1 nm Ch-b	0.186	0.124	0.369	0.224
A480 nm C x+c	0.360	0.218	0.669	0.451

Table-3 Quantification of Chlorophyll a, Chlorophyll b and Carotenoids (µg/ ml) of *Carica Papaya L.*in various chemical solvents

Plants	**Male leaves Kachchh**	**Female leaves kachchh**	**Male leaves Saurashtra**	**Female leaves Saurashtra**
Time	**15 min**	**15 min**	**15 min**	**15 min**
95 % Ethanol				
Ch-a	5.58	6.76	6.97	7.58
Ch-b	2.40	4.68	4.04	4.35
C x+c	2.12	2.00	2.93	2.21
Methanol				
Ch-a	7.08	3.23	4.43	1.83
Ch-b	4.25	1.97	1.69	1.1
C x+c	2.56	1.00	1.19	0.48
80 % Acetone				
Ch-a	6.34	5.68	4.96	8.24
Ch-b	3.28	3.63	2.08	4.66
C x+c	2.19	1.94	1.85	2.42
Diethyle ether				
Ch-a	4.95	4.39	3.09	8.84
Ch-b	1.63	2.3	1.38	5.73
C x+c	1.96	2.17	1.61	8.25
Dimethyl sulfoxide				
Ch-a	4.15	2.23	7.54	4.98
Ch-b	2.14	1.72	4.63	2.59
C x+c	1.09	0.56	1.86	0.82

Methanol was shown to have the highest extraction rate of carotenoids and chlorophylls (chlorophylls a and b) in the male *Carica Papaya L.* species from the Kachchh region. 95% ethanol demonstrates the maximum

extraction of chlorophylls (Chlorophyll a and b) and carotenoids for female *Carica Papaya L.* species from the kachchh area.

Dimethyl-sulphoxide (DMSO) exhibits the highest extraction of chlorophylls (Chlorophyll a and b) from the leaves of the male *Carica Papaya L.* Saurashtra species, while 95 %ethanol is the best solvent for carotenoids. The species chlorophylls (chlorophyll a and b) and carotenoids provide the greatest amount of extraction in diethyl-ether for female *Carica Papaya L.* from the Saurashtra region

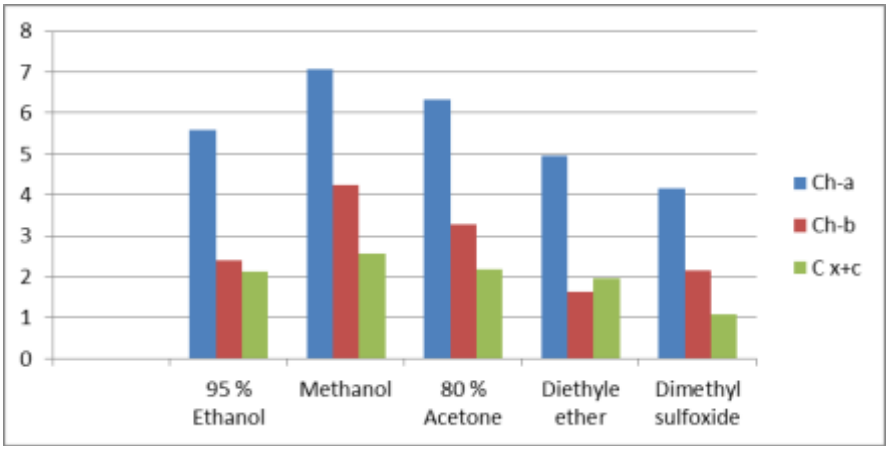

Fig. 1. The concentrations (µg/ ml) of Chlorophyll a, Chlorophyll b and Carotenoids in *Carica Papaya L.* Male leaves kachchh region

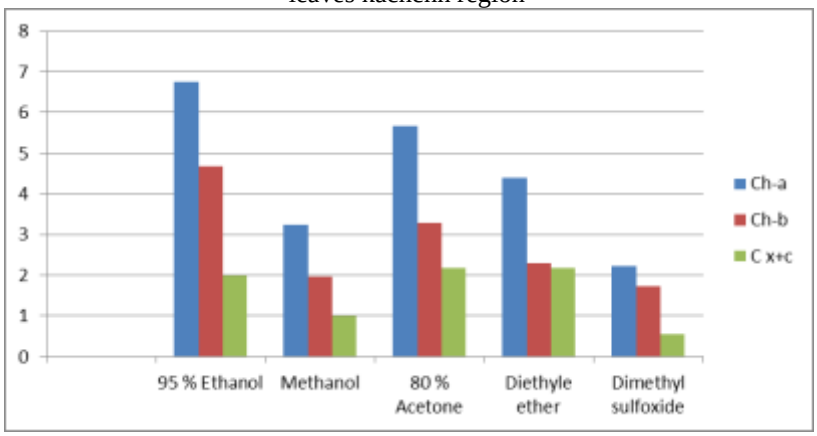

Fig. 2. The concentrations (µg/ ml) of Chlorophyll a, Chlorophyll b and Carotenoids in *Carica Papaya L.* Female leaves kachchh region

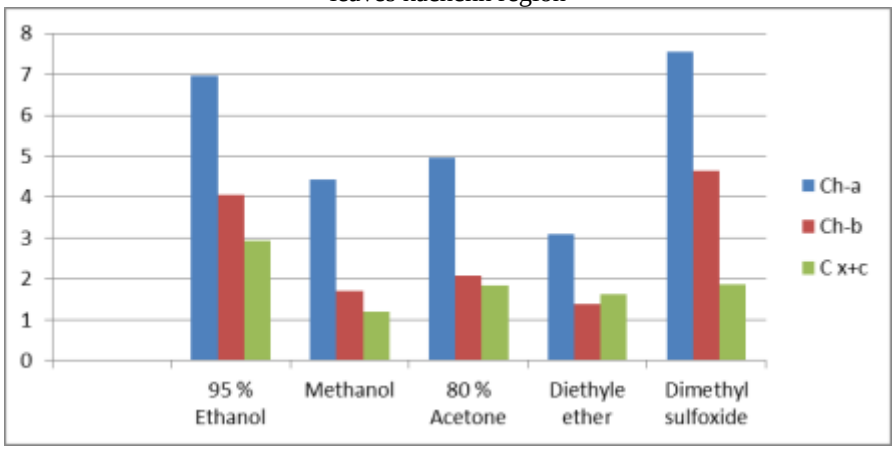

Fig. 3. The concentrations (µg/ml) of Chlorophyll a, Chlorophyll b and Carotenoids in *Carica Papaya L.* Male leaves Saurashtra region

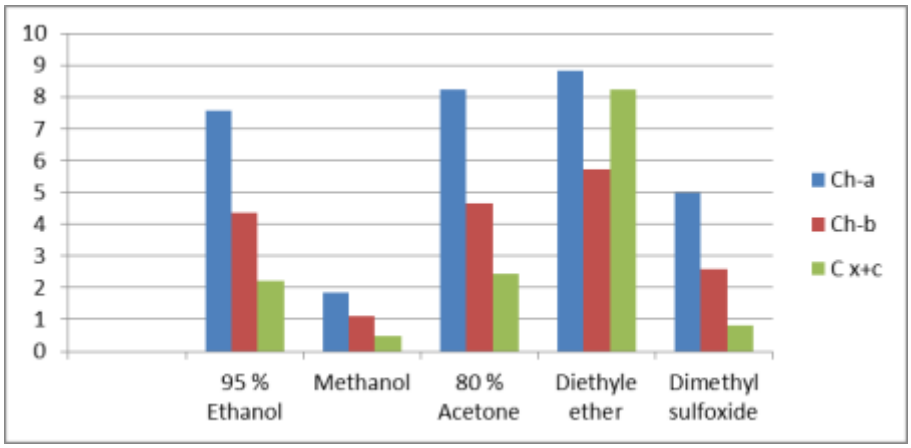

Fig. 4 The concentrations (µg/ml) of Chlorophyll a, Chlorophyll b and Carotenoids in *Carica Papaya L.* Female leaves Saurashtra region

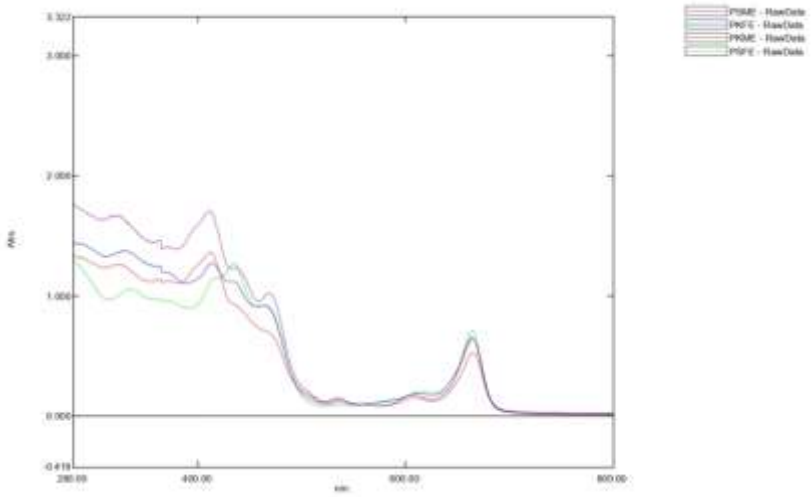

Fig. 5.UV spectroscopic determination of 95% ethanol

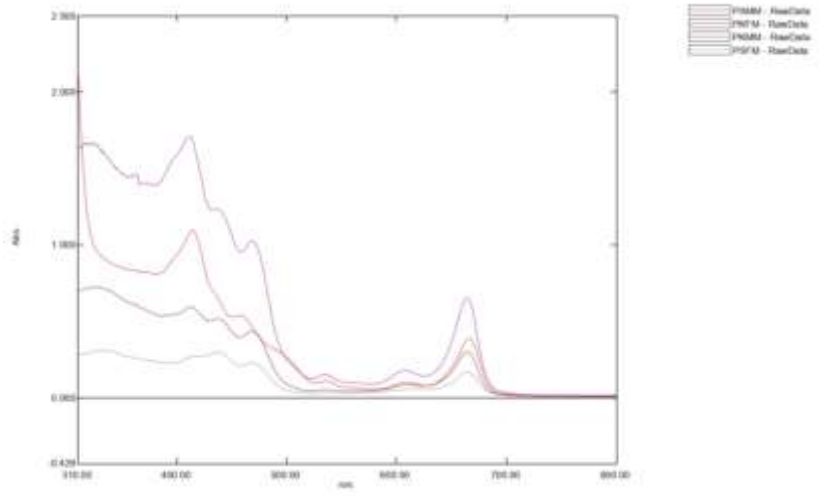

Fig.6. UV spectroscopic determination of methanol

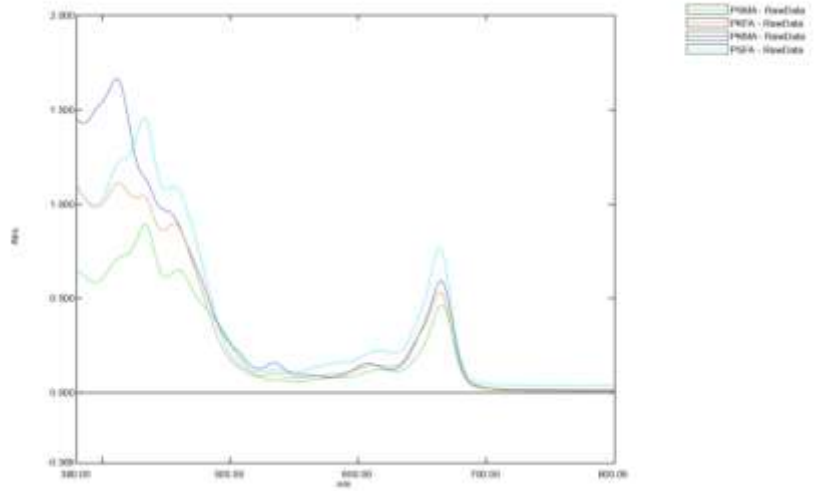

Fig.7. UV spectroscopic determination of 80% Acetone

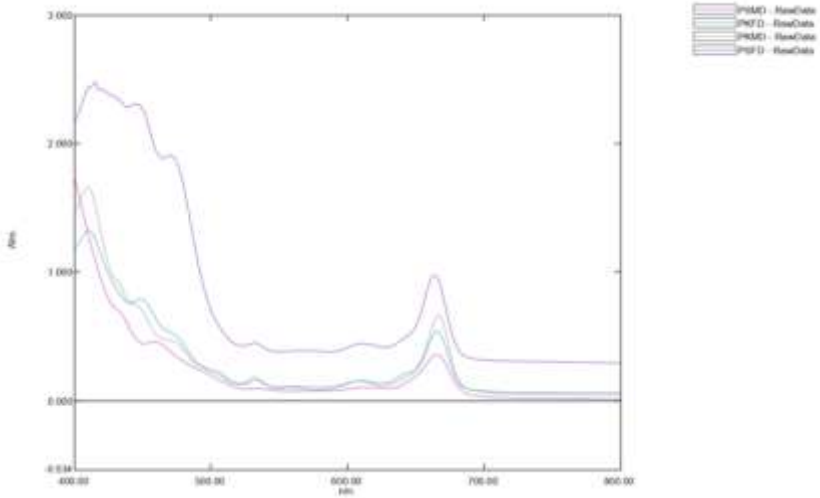

Fig.8 UV spectroscopic determination of Diethyle ether

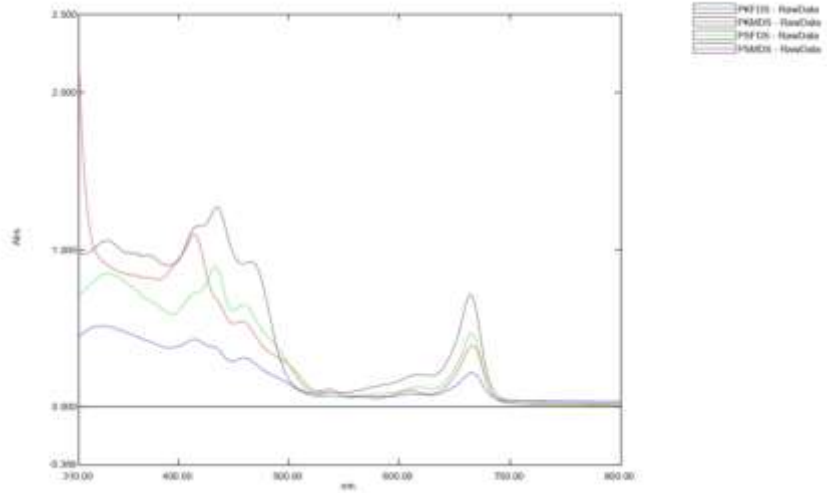

Fig.9 UV spectroscopic determination of Dimethyl-sulphoxide

IV. Discussion

The extractions of chlorophylls and carotenoids by different solvent for examined species are in the sequence of –

Male *Carica Papaya L.* kachchh region –

Chlorophyll-a: Methanol > 80%Acetone > 95% Ethanol > DEE> DMSO. **Chlorophyll-b**: Methanol > 80%Acetone > 95% Ethanol >DEE> DMSO. **Carotenoids**: Methanol > 80%Acetone > 95% Ethanol > DEE> DMSO

Female *Carica Papaya L.* kachchh region –

Chlorophyll-a: 95% Ethanol >80%Acetone > DEE > Methanol > DMSO **Chlorophyll-b:** 95% Ethanol >80%Acetone > DEE > Methanol > DMSO **Carotenoids:** DEE >95% Ethanol >80%Acetone > Methanol > DMSO

Male *Carica Papaya L.* Saurashtra region –

Chlorophyll-a: DEE > 95% Ethanol > 80%Acetone > Methanol > DMSO. **Chlorophyll-b**: DMSO> 95% Ethanol > 80%Acetone > Methanol > DEE **Carotenoids**: 95% Ethanol DMSO>80%Acetone> DEE> Methanol

Female *Carica Papaya L.* kachchh region –

Chlorophyll-a: DEE > 80%Acetone > 95% Ethanol > DMSO > Methanol **Chlorophyll-b**: DEE > 80%Acetone > 95% Ethanol > DMSO > Methanol **Carotenoids**: DEE > 80%Acetone > 95% Ethanol > DMSO > Methanol

Carotenoids are found in chromoplasts, which give vegetables and fruits their colour, as well as in chlorophylls, which function in conjunction with chlorophylls in both photosystems. About 70 chemicals belong to the carotenoids group and their derivatives, which are found in the majority of plants and fruits.[20] According to Vechetel and Ruppel, carotene pigments are the most significant photosynthetic pigments because they protect chlorophyll and the thylakoid membrane from oxidative damage caused by ingested energy. The extraction of carotenoids using different solvents from the species under study shows a tendency that is remarkably similar to the one seen for chlorophylls

V. Conclusion

The findings of this study demonstrate unequivocally that the chemical composition of biomolecules determines which solvents are best for extracting photosynthetic pigments (chlorophyll-a, chlorophyll-b and carotenoids). According to research, methanol is the best extraction solvent for extracting the largest amount of Ch-a, Ch-b, and carotenoids from male *Carica Papaya L.* leaves from the Kachchh region. In the ethanol solvent, the female leaves of *Carica Papaya L.* from the Kachchh region exhibit significant levels of Ch-a, Ch-b, and carotenoids. Male leaves of the *Carica Papaya L.* exhibit significant levels of Ch-a and Ch-b in DMSO solvent for the Saurashtra region sample. The highest levels of carotenoids are found in ethanol for the male *Carica Papaya L.* Diethyle ether is the best solvent and provides the most amount of extraction for female leaves in the Saurashtra region when it comes to Ch-a, Ch-b, and carotenoids. The present investigation found the greatest significant variations in the trend for pigment extraction in *Carica Papaya L.* reported before from phytoplankton and also from higher plants. Further research in this area is advised since variations in pigment concentrations in plants may potentially be caused by temporal and seasonal changes as well as local geological conditions.

References

[1]. Zeidali, Ehsan, et al. "Ethnopharmacological survey of medicinal plants in semi-arid rangeland in western Iran." Cent. Asian J. Plant Sci. Innov 1.1 (2021): 46-55.program (NCEP) expert panel on detection, evaluation, and treatment of highblood cholesterol in adults (adult treatment panel III) finalreport. Circulation. 2002;106(25, article 3143).

[2]. Chikezie, P. C., C. O. Ibegbulem, and F. N. Mbagwu. "Medicinal potentials and toxicity concerns of bioactive principles." Med Aromat Plants 4.3 (2015): 1-15.

[3]. Hishe, Mebrahtu, ZemedeAsfaw, and MirutseGiday. "Review on value chain analysis of medicinal plants and the associated challenges." Journal of Medicinal Plants Studies 4.3 (2016): 45-55.

[4]. Ramesh, Halavath, and M. F. Valan. "Rethinking on Using of Traditional Indigenous Medicinal Plants for the Management of Covid-19 In India-A Review." International Journal of Ayurveda and Pharma Research (2021): 53-63.

[5]. Balkrishna, Acharya, Pallavi Thakur, and AnuragVarshney. "Phytochemical profile, pharmacological attributes and medicinal properties of convolvulus prostratus–A cognitive enhancer herb for the management of neurodegenerative etiologies." Frontiers in pharmacology 11 (2020): 171.

[6]. Mykhailenko, Olha, et al. "Qualitative and quantitative analysis of Ukrainian Iris species: A fresh look on their antioxidant content and biological activities." Molecules 25.19 (2020): 4588.

[7]. Prasad, Anupama R., et al. "Applications of phytogenic ZnO nanoparticles: A review on recent advancements." Journal of Molecular Liquids 331 (2021): 115805.

[8]. Li, Penghui, and Jin Hur. "Utilization of UV-Vis spectroscopy and related data analyses for dissolved organic matter (DOM) studies: A review." Critical Reviews in Environmental Science and Technology 47.3 (2017): 131-154.
[9]. Morris, Rob. "Spectrophotometry." Current Protocols Essential Laboratory Techniques 11.1 (2015): 2-1.
[10]. Tian, Jian, et al. "Carbon quantum dots/hydrogenated TiO2 nanobeltheterostructures and their broad spectrum photocatalytic properties under UV, visible, and near-infrared irradiation." Nano Energy 11 (2015): 419-427.
[11]. Thyrhaug, Erling, et al. "Polarization and symmetry of electronic transitions in long fluorescence lifetime triangulenium dyes." The Journal of Physical Chemistry A 117.10 (2013): 2160-2168.Jones PH, Davidson MH, Stein EA, et al. Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR* trial) American Journal of Cardiology.2003;92(2):152–160.
[12]. Joshi, Shankar Gopal, and Shankar Gopal Joshi. Medicinal plants. Oxford and IBH publishing, 2000.
[13]. Van Vuuren, S. F., K. E. Motlhatlego, and V. Netshia. "Traditionally used polyherbals in a southern African therapeutic context." Journal of Ethnopharmacology 288 (2022): 114977.
[14]. DeFilipps, Robert A., and Gary A. Krupnick. "The medicinal plants of Myanmar." PhytoKeys 102 (2018): 1.
[15]. Porra, R. J., W. A. A. Thompson, and P. E. Kriedemann. "Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy." BiochimicaetBiophysica Acta (BBA)-Bioenergetics 975.3 (1989): 384-394.
[16]. Sumanta, Nayek, et al. "Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents." Res J Chem Sci 2231 (2014): 606X.
[17]. Lichtenthaler, Hartmut K., and Alan R. Wellburn. "Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents." (1983): 591-592.
[18]. Mirkovic, Tihana, et al. "Light absorption and energy transfer in the antenna complexes of photosynthetic organisms." Chemical reviews 117.2 (2017): 249-293.
[19]. Heidrich, Jennifer. Defined interactions are crucial for the structure and function of the Synechocystis IM30 protein. Diss. Johannes Gutenberg-Universität Mainz, 2018.
[20]. Costache, Manuela Adriana, G. H. E. O. R. G. H. E. Campeanu, and Gabriela Neata. "Studies concerning the extraction of chlorophyll and total carotenoids from vegetables." Romanian Biotechnological Letters 17.5 (2012): 7702-7708.

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