

Ultrasound-Assisted Extraction of Chlorophyll from Papaya Leaves And its Purification by Solid Phase Extraction A review.

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Abstract

Carica papaya L belongs to family of Caricaceae and well known for its remedial and beneficial nutritional effects. Leaves of *C. papaya* have been used for ages for the extraction of chlorophyll which is a good natural food colorant. Field of medicine is blessed with the advantageous biological composition and effects of wellbeing of *C. Papaya* plant. Many parts of this plant involving leaves, fruits and seeds are used in formation of Ethno medicines for the treatment of many infections and diseases including dengue fever. The Phytochemicals present in leaves are alkaloids such as pseudocarpain, carpain, dehydrocarpaine I and II. Some micro nutrients are vitamin C and E. Purpose of this study is to build up a process which is a combination of ultrasound-assisted and solid phase extraction (SPE). This process is intended to achieve the extract of chlorophyll with good yield along with acceptable extent of purity. Different variables including time, temperature, solvent's concentration and plant material were optimized separately during this stage of ultrasound

assisted extraction. Furthermore the attained extract was purified by passing through SPE and observed via UV-Vis spectrophotometer. The utmost yield of extract was found as 40% in optimized conditions of 80ml of ethanol, 20ml of water, extraction time (5) minutes, temperature (35 C°) and leaf powder 1gram. Moreover this purified sample was being examined by using UV-visible spectrophotometer and demonstrated the total substance of chlorophyll as (34mg/g), involving chlorophyll 'a' with concentration of (14.1246mg/g) and chlorophyll 'b' as (19.845mg/g). Therefore, ultrasound assisted extraction method can be recommended as a fine method to obtain enhanced chlorophyll content. Objectives of this Research work are to extract out natural colour from leaves by means of ultrasound assisted extraction technique, purification of attained colour from extraneous matter by means of Solid Phase Extraction, to obtain high yield of colour by this recently developed combination technique and to calculate chlorophyll content by means of UV-

Vis spectroscopy.

Keywords

Carica papaya L., Ultrasound assisted extraction, Solid phase extraction, Chlorophyll, UV-Visible spectroscopy.

1. INTRODUCTION

Carica papaya L commonly known as papaya or pawpaw is one of the most popular and commonly distributed plant of tropical region. Customarily it has been used for its dietary and therapeutic properties. Previous studies demonstrated remedial uses of various parts of *C.papaya* tree in the treatment of heart diseases, malaria, dengue, digestive problems, inflammations, wound healing, low blood sugar, lipid disorder, fungal and bacterial diseases (Pamdey *et al.*, 2017).

1.1. Chlorophyll in *Carica papaya*

Carica papaya is a very reasonable and exceptional source of natural bioactive substances including chlorophyll. Literature illustrated that leaves of *C.papaya* carries more content of chlorophyll as compared to other vegetables including borecole, curled mustard and spinach. Because of the exceptional nutritive report and better content of chlorophyll in *C.papaya* leaves, its extraction by using an appropriate method has become a requirement. Previously many traditional extraction methods have been used; however with the passage of time they have been replaced with the most recent techniques for the separation of phytochemicals (Mulgund, 2017).

1.2. Food colorants

Food colorant is an additive and not usually

considered a food, but also added to food products to give or re-establish color. Several consumables lose their color after passing through number of stages of processing and when they are stored, so the colorants could be helpful. Food colorants can be extracted out from several natural sources and can be produced as synthetic dyes. More preferable option is to produce the colorant by using natural source, because of their nutritional values. They not only accomplish the necessity of uniqueness but also enhance the food with nutritious components (Bajwa *et al.*, 2018).

1.3. Disadvantages of synthetic dyes

Food colorants are reported to be the cause of hyperactive behavior amongst children. Since synthetic food colors lack nutritional value, subsequently many adverse effects are rising badly to impact human health. Due to the consumer's requirement, manufacturers have started eliminating the artificial dyes from food and prefer the addition of natural color obtained from natural products.

Moreover, another matter of concern is the addition of illegal synthetic dyes with consumables. There are abundant dyes present which are used regardless of the fact that they are not good for human health (Oplatowska-Stachowiak *et al.*, 2017).

1.4. Benefits of natural color extraction

Substitution of synthetic dyes by natural colorants is gaining more consideration in the European Union, United States, and in other regions of the world. Beverages and food industries have been aggravated by consumer's growing requirement for natural food colorants.

Besides, there is a concern of some scientific researches on the possible health risk of artificial colors (Vinha *et al.*, 2018).

1.5. Extraction

Lately, researchers have been using new extraction methods which are also known as green extraction methods. These methods include Ultrasonic assisted extraction, Microwave-assisted extraction, pressurized liquid extraction, Supercritical fluid extraction and pressurized hot water extraction (Ameer *et al.*, 2017). Old traditional methods have so many drawbacks such as poor yield along with long-term degradation of bioactive compounds along with less adhering properties. Recently many new techniques are showing remarkable progress such as gamma radiation (Zia *et al.*, 2019). Ultrasound-Microwave assisted extraction technique which is a combination of Ultrasound and Microwave extraction has also been used by many researchers (Wizi *et al.*, 2018). In order to get reasonable extraction results, an ultrasonic bath is

more preferable as it encompasses a piezoelectric transducer and ultrasonic sounder. This method demonstrates the good performance at lesser temperature and averts the damage to thermally sensitive photochemical (Zia *et al.*, 2019). Ultrasonic bath has quite a lot of advantages like simple operation and it is also cost-effectively appropriate (Chemat *et al.*, 2017).

Consequently, a newly customized method is developed where two extraction methods i.e. Ultrasound-assisted extraction and solid phase extraction are combined in order to obtain high and pure yield of natural colorant chlorophyll from the leaves of *C. Papaya* plant. This method is not only cost effective but also includes favorable health effects, since Papaya leaf contains several health improving compounds (Zulqarnain *et al.*, 2021).

Table1. Extraction of chlorophyll from papaya leaves.

S.no	Methods	Chlorophyll content	References
1.	Stirring method.	74.804mg/L	Maulana <i>et al.</i> , 2015
2.	Cold extraction	29mg/g	Nissa <i>et al.</i> , 2015
3.	Soaking and stirring	3.1294 mg/ m ³	Rizali <i>et al.</i> , 2019

2. MATERIALS AND METHOD

The data was collected from Google Scholar, PubMed, Sci-Hub Finder and Web of Science, are summarized in result and discussion below.

3. RESULTS AND DISCUSSION

Maximum amount of extract was 40% in

the following extraction conditions with ethanolic solvent (80ml of ethanol and 20ml of distilled water), amount of raw material (1 gram), extraction time 5 minutes and the temperature 35°C of sonicator bath. Results demonstrated that extraction yield decreased with the increased temperature and time, meanwhile eth-

anol was better solvent.

The following results are in agreement with (Qadariyah *et al.*, 2019) in the extraction of lawsone (hennotannic acid) from leaves of Henna by using ultrasound-assisted extraction technique.

The carotenoids and chlorophyll are more non-polar while ethanol has more non-polar functional groups as compared to water (Rubab *et al.*, 2021). Hence the ethanol has high binding capacity for chlorophyll (Zhang *et al.*, 2020).

Extraction of gallic acid and catechin from *Syzygiumcumini* seed kernel by US- assisted extraction technique concluded that high temperature might have caused the destruction of desired bioactive component (Mahindrakar *et al.*, 2020). At the beginning most of the dye content has been extracted out already, but with the passage of time extraction ability was reduced (Ying *et al.*; 2011; Meng *et al.*; 2018).

As the temperature increases there is more probability of the destruction of desired bioactive component, hence the decrease in the amount of extract was observed (Ying *et al.*; 2011; Hu *et al.*, 2017). As a result, the suitable temperature for extraction was of 35 °C.

3.1. Chlorophyll content

Total Chlorophyll content was determined using UV-Vis spectrophotometer with absorbance at 663 nm and 644 and further quantified as chlorophyll a and b.

3.1.1. Total Chlorophyll content

Total chlorophyll content, was approximately 34mg/g.

The content of chlorophyll in *Carica papaya* L leaf was calculated as 74.80mg/L (Maulana *et al.*, 2015). Meanwhile, the concentration of chlorophyll in the foregoing re-

arch was reported as 34mg/g.

100% of solvent concentration of *Carica Papaya* leaves holds 3.1294mg/m³ chlorophyll (Rizaliet *al.*, 2019). In comparison to that, this work showed the extraction yield of chlorophyll was higher as 34mg/g.

4. CONCLUSION

Extraction method for natural drug is known to have ability to leach out every components through decoction and other means but some components need harsh treatments, i.e. Ultra temperature or acid exposure but ultrasound extraction and solid phase treatment for purification are brilliant techniques which are more preferable for chlorophyll and other colorants which are found in plants. In order to maintain safety, purity of extracts, high yield and securing high nutritional colorants and other contents from herbal extract, it is an excellent attempt. On the mentioned facts this proved to be a good research finding for future perspectives regarding extraction of obstinate colorants and chlorophyll in a safe and pure form with high yield in future.

The natural colorants are useful nowadays in the Pharma, Food, Cosmetic and Paint industries to replace the synthetic colorants by simple techniques.

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