Please cite this article as
Ravikumar. Reviews in Agricultural Science, 10: 304–327, 2022
https://doi.org/10.7831/ras.10.0_304

REVIEWS OPEN ACCESS

An Insight into Extraction, Isolation, Identification and Quantification of Bioactive Compounds from *Crataegus Monogyna* Plant Extract

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ABSTRACT

Natural products from medicinal plants provide a huge opportunity for the emergence of new drugs. *Crataegus monogyna*, also known as common hawthorn, is a medicinal shrub whose bioactive compounds gain importance worldwide because of its efficiency in treating chronic disease conditions. Due to increase in the need of these bioactive compounds, use of appropriate and standard analytical techniques holds a great demand. Analytical techniques include extraction, isolation, identification and quantification of bioactive compounds from *C. monogyna*. Numerous new methods have been established under each category of analytical techniques. However, standard methods for recovering, processing and utilising bioactive compounds remains a major gap both in pilot as well as industry levels. Therefore, the main aspect of this review is to exploit all analytical techniques employed on *C. monogyna*, highlighting critical parameters such as time, temperature, yield of extract, total phenolic content and antioxidant activity in order to enrich the production of antioxidant-rich bioactive compounds from *C. monogyna* plant extract.

Keywords

bioactive compounds, *C. monogyna*, extraction, identification and quantification

1. Introduction

Research on natural products such as medicinal plants and herbs was of incredible significance in the remedy of diseases since ancient times. In the past two decades, there was a drastic boom in the usage of herbal medicines as natural remedies for human ailments [1, 2]. One such herb with high nutraceutical potential is *Crataegus monogyna*, which is commonly known as single seeded hawthorn or common hawthorn. This herb falls under the largest genus *Crataegus*, belongs to the Rosaceae family and is endemic to Europe, Asia and Africa [3]. In the 1800s, Europe first documented hawthorn's positive effect in treating cardiovascular diseases. Hawthorn extracts had the ability to reduce the inclusiveness of inflammation, hypertension and thrombosis. Apart from that, *C. monogyna* is also used to treat kidney stones, digestive alignments, dyspnoea, cancer, nerve dysfunction and high cholesterol levels [3, 4, 5].

Bioactive compounds are divided into three main categories: (a) terpenes and terpenoids (approximately 25,000 types), (b) alkaloids (approximately 12,000 types) and (c) phenolic compounds (approximately 8,000 types) [6]. In

Received March 14, 2022, August 11, 2022, Accepted August 30, 2022
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C. monogyna, each part of the plant such as leaves, flowers, fruits, fruit peels, seeds, stems and branches have been extensively researched and proven to have beneficial effects in both in-vitro and in-vivo studies due to the presence of diverse bioactive compounds in them [7, 8, 9, 10, 11, 12, 13].

Plants generally produce a different type of compounds in different parts; hence, in *C. monogyna*, diverse bioactive compounds are present in each plant part. The presence of bioactive compounds plays an essential role in treating chronic diseases. According to European Pharmacopeia, *C. monogyna* leaves, flowers and fruits are accepted as potential medicine for curing various chronic disorders such as renal stone, cancer, high blood pressure and digestive ailments [14]. However, the application of appropriate analytical techniques for the utilisation of bioactive compounds effectively from *C. monogyna* in treating chronic disease conditions gains high priority.

Analytical procedures include extraction, isolation, identification and quantification of bioactive compounds [15]. As mentioned above, plant parts of *C. monogyna* contain various bioactive compounds contributing to the bioactivity; however, the bioactivity may differ in efficacy based on the type of bioactive compound present and analytical procedure employed on them [16]. Previously, different plant parts of *C. monogyna* were studied and subjected to analytical processes in order to characterise different types of bioactive compounds present in them. Therefore, the main scope of this review paper is to analyse the analytical techniques applied on *C. monogyna* along with its critical parameters and its effect on biological properties.

Among all analytical techniques, extraction is the primary step in the processing of bioactive compounds [6]. Conventional and non-conventional extraction techniques have been used to extract bioactive compounds from medicinal plants [17]. Non-conventional extraction techniques such as Ultrasound Assisted Extraction (UAE), Microwave Assisted Extraction (MAE) and Supercritical Fluid Extraction (SFE) are widely applied in the industry when compared with conventional extraction techniques, such as maceration, infusion, digestion, decoction and percolation. This is due to low-cost production by minimal use of solvent and raw materials, with high efficiency of bioactive compound recovery [18, 19]. Therefore, to ensure that the bioactive compounds derived from *C. monogyna* are economically viable and remain competitive, an extraction method that could preserve the bioactivity and give high yield is required.

The efficiency of the extraction technique depends on the extraction parameters (e.g., time, temperature, solvent type), parts of the *C. monogyna* plant and the chemical nature of the bioactive compound to be recovered. Thus, the efficiency, advantages and disadvantages of various extraction conditions and techniques—such as Soxhlet, maceration, SFE, MAE and UAE that have been applied for extraction of bioactive compounds from *C. monogyna* at lab scale and pilot—will be reviewed in this paper.

In any analytical processing, after extraction, the separation of compounds plays an essential role in identification of typical properties of group or individual compounds. Separation of compounds, identification and characterisation of bioactive compounds are challenging as the plant extract usually occurs as a combination of various types of bioactive compound with different polarities [20]. Therefore, separation of different bioactive compounds from *C. monogyna* and identifying its biological activity remains a major gap.

In relation with biological activity, in the *C. monogyna* plant extract, various clinical trials portrayed reduced symptoms of congestive heart failure and improved cardiac performance classified using the New York Heart Association (NYHA class II) [10, 21, 22, 23]. Therefore, antioxidant activity of bioactive compounds from the extraction and separation process could be analysed by means of isolating individual compounds using chromatographic techniques.

Based on past findings, phenolic compounds from *C. monogyna* plant extract are the main contributor of antioxidant activity, including free radical scavenging activity [8, 24, 25, 26, 27]. However, it is necessary to isolate

individual compounds from *C. monogyna* plant parts with high antioxidant activity to understand the potential effect of each compound with respect to their antioxidant activity. This review summarises various chromatographic techniques such as thin layer chromatography (TLC), column chromatography (CC) and high-performance liquid chromatography (HPLC) which have been used for the isolation of bioactive compounds from the *C. monogyna* plant.

This directly aids in understanding the advantages and disadvantages of each chromatographic technique, along with its strength, limitation and precautionary steps from which most effective methods for the separation of a single pure antioxidant-rich bioactive compound could be devised. Moreover, effective separation and isolation techniques remain under mandatory criteria, since they influence the subsequent processing technique, such as structural elucidation and other ensuing techniques.

Structure elucidation or identification of the compound aids in understanding the chemical composition of the compound, which has a crucial role in analysing the type and biological activity of the bioactive compound. The concept of structural elucidation in relation with bioactivity was first identified by Crom-Brown and Fraser in 1865. This concept was refined by mathematical relationships such as Quantitative Structure-Activity Relationship (QSAR) and Safety Assessment and Feasibility Interim Report (SAFIR) [28]. Spectroscopic analysis namely, mass spectroscopy (MS), Fourier transform infrared (FTIR) spectroscopy and nuclear magnetic resonance (NMR) were used to identify the bioactive compound in *C. monogyna* plant extracts [29, 30, 31, 32].

As mentioned above, identification of the single pure antioxidant-rich compound aids in the field of drug discovery for chronic diseased conditions. After identification of the bioactive compound, the process of evaluating the concentration or percentage of structure elucidated compound is quantification. Quantification of the isolated, identified and structure elucidated compound is required to relate the activity of the bioactive compound with respect to its quantity [33, 34].

Thus, the main aim of this review paper is to critically summarise different extraction techniques employed on the *C. monogyna* plant with special attention to the type of solvent used for extraction along with the type of bioactive compounds extracted from each solvent type. Secondly, separation and isolation techniques employed on the *C. monogyna* plant with its future prospects are discussed. Thirdly, structural elucidation and quantification techniques employed on *C. monogyna* are discussed in relation to the single pure antioxidant-rich bioactive compound. Hence, the overview of this review paper is on the analytical techniques (such as isolation, identification and quantification) with respect to bioactive compounds from *C. monogyna* plant extract. The advantages and potential gaps of all the techniques are also highlighted.

2. Antioxidants extracted from different plant parts of *C. monogyna*

C. monogyna plant is loaded with bioactive compounds. By reviewing each plant part, that part with high antioxidant activity can be identified and it's more efficient to study the antioxidant activity of individual compounds, which in turn lays a platform in the identification of new compounds that till date remains a major gap. Table 1 shows the different bioactive compounds extracted from leaves, flowers, fruits, fruit peels, seeds, stems and branches of *C. monogyna*.

From Table 1, fruits, flowers and leaves of *C. monogyna* are widely researched by many researchers when compared with other plant parts such as branches, stems and seeds. This indicates that *C. monogyna* leaves, flowers and fruits provide an abundant source of bioactive compounds when compared to all other parts [14]. Majority of bioactive compounds in *C. monogyna* are phenolic compounds such as hyperoside, vitexin, vitexin-2"-*O*-rhamnoside,

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https://doi.org/10.7831/ras.10.0_304

vitexin-4"-*O*-rhamnoside, cyanidins, tocopherol (α, β, γ and δ), rutin and chlorogenic acid. Besides that, ascorbic acid and β-carotene are also present in *C. monogyna* plant extract.

The structure of each compound is shown in Fig.1. Extracts containing hawthorn constituents that have been marketed include Crataegutt®, Crataemon, Cardiplant®, Phyto H Complex, Crataegisan, WS® 1442, LI 132. These extracts are constituted of typical polar plant constituents such as flavonoids, triterpenic acids and oligomeric procyanidins, which are considered as the main constituents implicated in the prevention of congestive heart failure and other cardiovascular diseases. Flavonoids and condensed tannins extracted from *C. monogyna* are proven to have vasodilation, antiarrhythmic and hypotensive effects [23, 43, 44].

Table 1: Bioactive compounds extracted and identified from different parts of *C. monogyna* plant

C. monogyna plant part	Extracted and identified bioactive compounds	Sources
Leaves	Hyperoside, vitexin and vitexin-2″-o-rhamnoside	[9]
	Vitexin-4″-o-rhamnoside, apigenin, cyanidin	[8]
Flowers	Hyperoside, vitexin and vitexin-2″-o-rhamnoside	[9]
	Tocopherol (α, β, γ and δ), ascorbic acid, β-carotene	[35]
Flower buds	Tocopherol (α, β, γ and δ), ascorbic acid, β-carotene	[35]
Unripe fruits	Tocopherol (α, β, γ and δ), ascorbic acid, β-carotene	[35]
Ripened fruits	Tocopherol (α, β, γ and δ), ascorbic acid, β-carotene	[35]
Overripe fruits	Tocopherol (α, β, γ and δ), ascorbic acid, β-carotene	[35]
	Chlorogenic acid, hyperoside, rutin, quercetin, vitexin-2″-o-rhamnoside, epicatechin, catechin, procyanidin b2	[36]
	Vitexin-4″-o-rhamnoside, apigenin, cyanidins, chlorogenic acid	[37]
	Proanthocyanidine, cyanidins, quercetin, rutin, apigenin	[38]
Fruit peel	Proanthocyanidine, cyanidins, quercetin, rutin, apigenin	[38]
Fruit seed	Proanthocyanidine, cyanidins, quercetin, rutin, apigenin	[38]
Top branches	Hyperoside, vitexin and vitexin-2″-o-rhamnoside	[39]
Stem	Vitexin-4″-o-rhamnoside apigenin, cyanidins, chlorogenic acid	[38]

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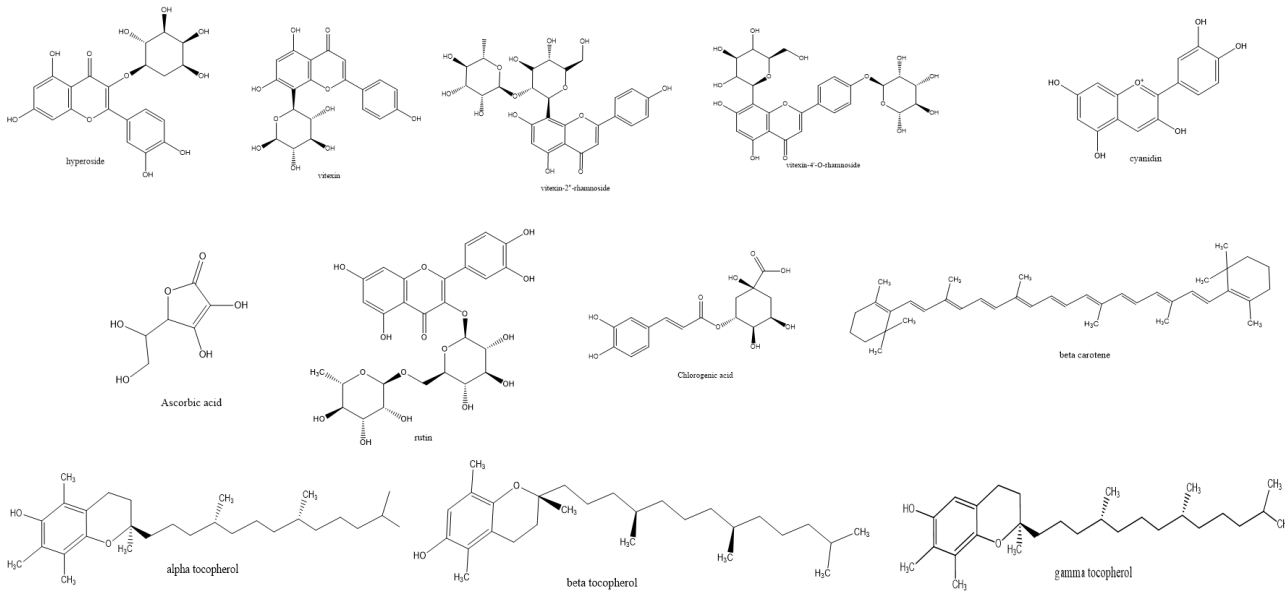

Figure 1: The general structure of bioactive compounds extracted and identified from *C. monogyna*

The bioactive compounds hyperoside, vitexin, vitexin-2"-*O*-rhamnoside, vitexin-4"-*O*-rhamnoside apigenin, cyanidins, chlorogenic acid and rutin present in *C. monogyna* have a direct as well as indirect relationship in treating cardiovascular diseases [42]. These compounds were reported to have antioxidant, anti-hyperglycaemic, anticancer, anti-inflammatory and anticoagulant activities [43]. These bioactive compounds have established an attenuated pre-treatment in vascular inflammation induced by high-glucose levels in both in-vitro and in-vivo studies. In the in-vitro studies, the protective mechanism for ischemic heart disease by significantly reducing reactive oxygen species (ROS) levels, improving mitochondrial activity, mitochondrial membrane potential and adenosine triphosphate (ATP) content. Markedly increasing mytofusin-2 (MFN2) expression and reducing the recruitment of dynamin related protein 1(Drp1) in mitochondria, protection of endothelial cells against hypochlorous acid (HOCl) induced oxidative damage [47, 48, 49, 50, 51, 52, 53, 54, 55].

In leaves, different bioactive compounds, especially phenolic acids and flavonoids, have been identified (Table 1). Phenolic compounds like hyperoside, vitexin, vitexin-2"-*O*-rhamnoside, vitexin-4"-*O*-rhamnoside, chlorogenic acids and cyanidins were extracted from leaves. *C. monogyna* leaves are one of the finest sources to extract bioactive compounds. Literature states each isolated component possesses various free radical scavenging activity [53], which is commercially used as a natural antioxidant for treating various types of illness and diseases. Thus, bioactive compounds extracted from *C. monogyna* leaves could be a potential source for manufacturing natural antioxidants.

C. monogyna flowers are also an excellent source of bioactive components. However, the usage of flower and flower buds for the extraction of bioactive compounds seems very limited. Bioactive components like hyperoside, vitexin, vitexin-2"-*O*-rhamnoside, tocopherols (α, β, γ and δ), ascorbic acid and β-carotene were extracted from flowers [35]. Tocopherols (α, β, γ and δ), ascorbic acid and β-carotene were extracted from flower buds.

Fruits of *C. monogyna* have an abundant source of bioactive compounds (Table 1). Polyphenols are the major source of bioactive compounds obtained from the small red fruits of the hawthorn plant [36]. Hawthorn fruit extracts could inhibit the oxidation of low-density lipoprotein (LDL) [40, 41, 57]. Due to increased interest in fruit consumption and growing of its products, there is a positive effect on fruit polyphenols on human health [57, 58].

Apart from polyphenols, bioactive components such as chlorogenic acid, epicatechin and hyperoside are also present in the fruit and contribute to the free radical scavenging and antioxidant activities of hawthorn fruit [35, 56]. Higher antioxidant activity from hawthorn fruit was contributed by phenolics and proanthocyanidins classes, whereas negligible amount of antioxidant activity was contributed by flavonoids and anthocyanins [34].

Parts of fruits like peels and seeds were used for extraction; extractions were also made from unripe, ripe and over-ripened fruits (Table 1) in order to isolate bioactive compounds for its antioxidant properties. Bioactive compounds like tocopherol (α, β, γ and δ), ascorbic acid, β-carotene, cyanidins, quercetin, rutin and apigenin were isolated from unripe, ripe and over-ripened fruits [38]. Fruit berries contain flavonoids such as chlorogenic acid, hyperoside, rutin, quercetin, vitexin-2"-*O*-rhamnoside, epicatechin, catechin, procyanidin B2 and cyanidins. Fruit peels and seeds of *C. monogyna* contain bioactive compounds belonging to the classes of proanthocyanidins, anthocyanin, flavonols and flavones [57].

Besides the plant parts mentioned above, the top branches and stem were also used for extraction of bioactive compounds. Compounds like hyperoside, vitexin, vitexin-2"-*O*-rhamnoside, vitexin-4"-*O*-rhamnoside apigenin, cyanidins and chlorogenic acid were identified (Table 1). Therefore, from Table 1, plant parts of *C. monogyna* extracts are good sources of bioactive compounds; fruits, flowers and leaves are potent sources of bioactive compounds when compared with peels, seeds, branches and stem. The usage of these plant parts is also less because of their acceptability and *C. monogyna* leaves, fruits and flowers are widely accepted by most of the pharmacopoeia [14].

Therefore, different plant parts of *C. monogyna* have been used for extraction. However, the major gap is to identify the particular *C. monogyna* plant part which yields high antioxidant activity. From the usage of *C. monogyna* plant part and the number of researches conducted concludes that the hawthorn fruits are considered to have a promising source for high antioxidant bioactive compounds extracted and identified from them (Table 1), which could be further analysed by comparing bioassays such as 2,2-diphenyl-1-picrylhydrazy (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferric reducing antioxidant power (FRAP).

The main advantage of the identification of plant parts is that it serves as a platform for various other researches such as in the field of drug discovery for the isolation of targeted bioactive compound from the antioxidant-rich plant part of hawthorn. In other perspectives, identification of plant part remains as a base through which further analytical techniques such as isolation, identification and quantification can be carried out in an effective manner.

3. Extraction techniques employed on *C. monogyna*

In general, plant metabolites are classified as primary and secondary metabolites. Primary metabolites such as sugars, proteins and fats are found in plants. The secondary metabolites are essential for antagonistic interactions, which are collectively called as bioactive compounds or phytochemicals [58]. These phytochemicals or bioactive compounds are not required by plants for their basic function. However, these compounds provide a valuable bioactive interaction for humans in order to overcome various degenerative disorders like hypertension, kidney stones, digestive ailments, dyspnoea, hyperlipidaemia and so on [59]. Hence, to use those bioactive compounds from the *C. monogyna* plant, the finest way to utilise them is by different extraction techniques.

Extraction is a predominant step in the analytical processing of plants. Both conventional and non-conventional methods have been applied for the extraction of bioactive compounds from the *C. monogyna* plant [60]. Conventional methods such as Soxhlet and maceration and non-conventional methods such as supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) have been applied for the extraction of bioactive compounds from *C. monogyna*. A detailed explanation on the extraction techniques

(conventional and non-conventional) typically applied for extraction of bioactive compounds, along with the effect of parameters such as extraction time, temperature and solvent type on the extraction yield are explained in detail.

3.1 Extraction techniques

3.1.1 Soxhlet extraction

In the hawthorn plant, Soxhlet extraction was carried out with *C. monogyna* leaves and flower powder, to extract phenolic compounds such as hyperoside, vitexin and vitexin-2"-O-rhamnoside. On comparing Soxhlet extraction with other non-conventional extraction techniques, the extract yield of *C. monogyna* leaves and flowers was comparatively low in the Soxhlet extraction method [9]. Soxhlet extraction is a conventional technique commonly used for the extraction of bioactive compounds. Soxhlet extraction is usually performed in a Soxhlet apparatus; 'thimble' is made of strong filter paper or cellulose, where the sample is placed, and the extraction process is carried out. The basic principle in Soxhlet extraction is that the extraction solvent is heated at the bottom flask, which again vaporises into the sample placed in the thimble. The process of condensation takes place, due to which the liquid contents get emptied into the bottom flask again and the process is continued [61].

The advantages of using the Soxhlet extraction technique is less expensive, the temperature in the extraction system can be maintained and it is a mechanically gentle process. Hence, it does not require any complicated instrumental handling. However, Soxhlet extraction requires more solvent when compared to other non-conventional extraction techniques [62]. The Soxhlet extraction technique is not an environment-friendly method due to the heating of hazardous flammable organic solvents that could lead to the emission of toxic fumes during extraction [65, 66]. The major factors that affect the extraction yield are solvent-sample ratio and temperature of extraction, which stills remains a critical point. Moreover, decomposition of heat-sensitive bioactive compounds is high when compared with other non-conventional methods of extraction.

3.1.2 Maceration extraction

For maceration extraction, different plant parts of *C. monogyna* like leaves, flowers, fruits and a combination of them were subjected to extraction for bioactive compounds. Maceration was initially used for the manufacturing of wine and then later, it was also adapted for the extraction of bioactive compounds from medicinal plants. The basic principle of the maceration process is to soften and break the plant cell wall, which helps in releasing the phytochemicals. Typically, the plant material to be extracted is allowed to soak in extraction solvent (aqueous, ethanol and methanol) for about 48–72 hours. In this conventional method, heat is transferred through convection and conduction for the extraction of desired bioactive compounds [64, 65].

From researches, maceration requires more time for extraction based on the type of compound to be extracted [9, 31, 67, 68]. When comparing the total phenolic content (TPC) with other non-conventional extraction techniques, the TPC obtained from maceration is typically low. This is due to prolonged extraction time leading to degradation of bioactive compounds. Among other conventional techniques, maceration is considered as the simplest and easiest method of extraction. However, when considering the point of prolonged extraction time, low yield, less stable bioactive compound and usage of more volume of extraction solvent, the maceration technique has more organic waste when compared with other extraction techniques, and hence, this remains a major limitation [31, 57, 67].

3.1.3 Supercritical fluid extraction

Supercritical fluid extraction (SFE) falls under the category of non-conventional extraction techniques. Hawthorn plant parts such as leaf, flower and fruit extracts was subjected to SFE (static extraction and dynamic

extraction) with CO_2 flow rate of 5 L/min with methanol as the extraction solvent; overall, the results stated that the TPC, DPPH and FRAP of hawthorn leaf, flower and fruit extract was higher than the values obtained from conventional techniques [65]. Currently, SFE is used in different fields such as food science, natural products, by-product recovery, pharmaceutical and environmental sciences [68].

Supercritical fluid extractions were performed using a speed SFE system. The system was fitted with a 24 mL stainless-steel extraction vessel. The most critical point of this extraction is the physical property of gas and solvent [69]. Using SFE as the extraction technique, the main advantage is that excellent solvent for non-polar analyte and carbon-dioxide is abundantly available and it's less expensive. But the major drawback is that the supercritical carbon-dioxide has poor solubility towards polar compounds, therefore modification of parameters such as solvent type and temperature could help in recovering a few of the polar compounds through SFE.

The extraction time of SFE is minimum because of its adaptability of altering the pressure and temperature. However, the initial cost of the supercritical fluid equipment still remains a major drawback since it is more expensive; another drawback is added training to control and maintain the equipment's parameters [72, 73].

3.1.4 Ultrasound-assisted extraction

Sonication of hawthorn leaf, flower and fruit extract revealed high TPC, DPPH and FRAP activity. UAE involves the use of ultrasound waves for extraction, typically 20–100 kHz, with a sound intensity ranging from 10 to 1000 W/cm^2 for the extraction of bioactive compounds [72]. The basic principle behind UAE is the acoustic cavitation of the bubbles between the sample and solvent; this disturbance results in high acceleration, breaking and mixing the plant material, evolving into increased plant molecular motion and speed, finally increasing the solvent penetration. This action results in increased permeability of bioactive compounds from the plant cell wall into the extraction solvent. This method helps in enhancing the mass transport of solvents into the plant cells; hence, UAE is widely used in both small as well as large-scale industries for the extraction of bioactive compounds [73].

UAE is also considered as a simple non-conventional extraction technique and it is more efficient in terms of time, cost, usage of solvent and recovery of the extract; UAE is also named as "Green Extraction Method" [65, 76, 77]. Therefore, the advantages of using UAE are less time- and solvent-consuming, increased yield and high quality of the extract obtained [68]. The major limitation of using UAE as an extraction medium is the consequence of the degrading effect of certain active bioactive compounds by the formation of free radicals when using higher frequencies [61, 75].

3.1.5 Microwave-assisted extraction

Microwave-assisted solvent extraction has been applied for the extraction of hyperoside, vitexin and vitexin-2 "-*O*-rhamnoside from various parts (branches, flowers and leaves) of *C. monogyna*; nevertheless, other major criteria like extraction yield, TPC, DPPH, ABTS and FRAP were not reported [9, 76]. Microwave energy is used to facilitate the MAE process. The principle behind MAE is the microwave generated interacts with the dipoles of the solvent and sample matrix, resulting in heating the matrix, where heat is transferred through conduction. MAE favours the extraction of more polar molecules, and solvent with high dielectric constant is used for extraction. Because of the dipole rotation of the molecules caused by microwave, the electromagnetic force disrupts hydrogen bonding, which finally enhances the migration of the dissolved ions and promotes solvent penetration into the sample matrix [80, 81].

The major advantage of MAE is the reduced extraction time and solvent when compared to other conventional extraction techniques. The major disadvantage of MAE is the occurrence of thermal degradation. Hence, this method

is limited to small-molecular phenolic compounds such as gallic acid, ellagic acid (phenolic acids), quercetin, isoflavones and trans-resveratrol. These molecules are highly stable under microwave heating conditions up to 100°C for about 20 minutes [78]. However, when comparing the yield of total phenols and flavonoids, there is a drastic decrease due to oxidation of the bioactive compounds by increasing the cycle of MAE (e.g., from 2×10s to 3×10s).

Therefore, to summarise, MAE is not technically suitable for extraction of heat-sensitive bioactive compounds which are subjected to degradation at high temperature (e.g., tannins and anthocyanins) [64, 80, 81]. However, all the extraction techniques mentioned above used individual parts of *C. monogyna*. From all the studies reviewed, there is a lack of research to identify the *C. monogyna* plant part which has the highest total phenolic content and antioxidant activity, which should be experimented in future studies.

Comparing these five extraction techniques applied on *C. monogyna*, ultrasound-assisted extraction (UAE) is recommended on the base of its ease of use, robust processing, less solvent consumption and minimum time duration with efficient extraction of a large amount of desired bioactive compounds. Recently, UAE was also termed as "Green Method" because of its vast advantages. The *C. monogyna* extracts obtained by ultrasound-assisted extraction (UAE) method exhibited high antioxidant property (3082.00 ± 13.58 mg AAE/l DPPH, 1630.99 ± 8.99 mg AAE/l FRAP) [65].

The other methods discussed were effective for the extraction of bioactive compounds but exhibited low efficiency, difficulty in handling, more processing steps than UAE [73]. From comparing the extraction techniques, it is seen that researchers have been searching for the potential methods which could result in higher extraction yield, better selectivity, less solvent consumption and energy requirement, fast extraction time, as well as eco-friendly processes and finally, to possess high antioxidant activity of the extracts, which is covered under UAE [80, 81].

Critical parameters such as temperature, time and type of solvent used for extraction also play a major role in the extraction process. Solvent used for extraction determines the rate of extraction; the type of bioactive compound to be extracted also determines the biological activity such as antioxidant property of the extract. Hence, the impact of solvent on extraction in the *C. monogyna* plant extract is reviewed in the following section.

3.2 Impact of extraction solvent on *C. monogyna* plant extract

Solvent plays a critical role in the extraction of bioactive compounds. Majority of chemical reactions take place in the liquid phase. At the macroscopic level, a liquid is an ideal medium to transport heat side to side in both exo and endothermic reactions. A liquid medium which facilitates the reaction is called as solvent [82]. Solvents aid the blending of plant material to regulate temperature, stabilise (or destabilise) reactive intermediates and mediate proton transfers. The specific choice of solvent can make the difference between success and failure of a reaction [83].

Different solvents provide exclusive chemical substances. Polar solvents (e.g., water, ethanol and methanol) are usually applied as the extraction solvent for the extraction of hydrophilic phenolic compounds while non-polar solvent (e.g., hexane, chloroform, petroleum ether) are used for the extraction of hydrophobic substances such as oil and fat [84].

Previous studies on *C. monogyna* have used methanol, ethanol and water as solvents for extracting bioactive compounds for further analysis. Solvents for extraction were selected based on their polarity index. The solvent polarity index for water, methanol and ethanol are 9, 6.6 and 5.1, respectively [85]. Although various solvents have been applied to extract numerous bioactive compounds from *C. monogyna*, none of the previous studies has compared the impact of commonly used solvents on the extraction efficiency of phenolic compounds from the *C. monogyna* plant.

Please cite this article as
Ravikumar. Reviews in Agricultural Science, 10: 304–327, 2022
https://doi.org/10.7831/ras.10.0_304

Table 2 demonstrates different types of polar and semi-polar solvents employed on *C. monogyna* plant parts for extraction, along with their targeted activity and type of instruments with concentration of analytical solvents used. The main aim of this table is to understand the role of solvent in the extraction of *C. monogyna* plant part and its influence in certain targeted activity starting from the extraction of bioactive compounds till cell line studies.

Table 2: Solvents employed on *C. monogyna* plant part and targeted activity

C. monogyna plant part	Extraction technique	Solvent used	Targeted activity	Instrumentation	Source
Leaves	Solvent extraction (mortar and pestle)	70% methanol	Phenolic compounds extraction (epicatechin, catechin, chlorogenic acid, vitexin, vitexin-2"-*O*-rhamnoside, acetylvitexin-2"-*O*-rhamnoside, hyperoside, quercetin, and rutin)	Phenomenex luna^{tm} column [5 mm pore size, C-18, 150mm×4.60mm]; HPLC analysis Solvent A- water + 0.1% + TFA (trifluoroacetic acid) Solvent B- acetonitrile + 0.1% + TFA	[86]
Flowers, leaves and fruits	Solvent extraction (mortar and pestle)	Ethanol and water	Antioxidant activity (H_2O_2 scavenging activity and inhibition of lipid peroxidation activity)	UV-Vis spectrophotometer	[87]
Top branches, leaves and flowers	Soxhlet, maceration, ultrasound and microwave-assisted extraction	Ethanol	Phenolic compound extraction (hyperoside, vitexin and vitexin-2"-*O*-rhamnoside)	Phytocomponents identified and quantified by HPLC-UV/PAD	[9]
Leaves and stem	Maceration	Ethanol	Atropine-sensitive activity in a cultured cardiomyocyte assay	Size- exclusion chromatography (LH-20 resin)	[88]
Leaves	Maceration	Methanol	Phenolic compound extraction (flavonol [(-)- epicatechin] and flavonoid (vitexin 2"-*O*-rhamnoside, acetylvitexin 2"-*O*-rhamnoside, and hyperoside)	HPLC analysis	[89]
Callus	Maceration	Absolute methanol	Identification of total phenolic content, total proanthocyanidine content, total anthocyanin content and total flavonoid content	Assays based on the Trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP) and stability in oil-in-water emulsion were used to characterise the antioxidant actions of the callus cultures	[90]
Fruit	Percolation	Aqueous and ethanol	Flavonoids (chlorogenic acid, hyperoside, rutin, quercetin, vitexin-2″-*O*-rhamnoside,	HPLC spectrophotometry	[91]

			epicatechin, catechin, and procyanidin B2). Antioxidant activity (DPPH and ABTS)		
Leaves	Maceration	Methanol	Procyanidins	Organ chamber studies	[13]
Leaves and sprouts	Decoction	Aqueous ethanol	Flavonoids	Capillary zone electrophoresis (CZE) HPLC	[33]
Leaves, flowers and berries	Maceration, sonicated extraction and supercritical fluid extraction	Ethanol	Measurement of lipid oxidation and oxymyoglobin oxidation	2-thiobarbituric acid-reactive substances (TBARS) and double- beam spectrophotometer	[65]
Fresh fruits, commerciall y dried fruit, flowering tops (flowers with young leaves) and flower buds	Maceration	Ethanol	Extraction of phenolic compounds (rutin, quercetin, epicatechin, hyperoside, chlorogenic acid, procyanidin B2)	Thin-layer chromatography (TLC) analysis, Shimadzu apparatus (LC-10AS pumps, SCD-10A detector, SCL-10Avp controller, LC solution software	[32]
Fruit (peel, pulp and seed)	Ultrasound-assisted extraction	Methanol	Extraction of proanthocyanidine, anthocyanin, flavonols and flavone compounds (5-*O*-caffeoylquinic acid, protocatechuic acid, Procyanidin trimer C1, Procyanidin dimer B2, Chlorogenic acid, Procyanidin trimer C2, Cyanidin-3-*O*-glucoside, (-)-epicatechin, Luteolin-7-*O*-rutinoside, Cyanidin-3-*O*-arabinoside, Quercitin-3,5-*O*-digaluctoside, Quercitin-3,5-*O*-diglucoside, Kaempherol-3-*O*-galactoside, Kaempherol-3-*O*-glucoside, Apigenin-7-*O*-glucoside, Quercitin-3-*O*-galactoside, Quercitin-3-*O*-glucoside)	HPLC-UV HPLC-MS UV-spectra	[25]
Fruit	Ultrasound-assisted extraction	Water hydro-alcohol (methanol), alcohol (ethanol), absolute methanol and	Identification of total polyphenols, Total flavonoids, total monomeric anthocyanins, total proanthocyanidins and antioxidant activity (DPPH)	Spectrophotometric method	[34]

		absolute ethanol			
Fruit and aerial part (leaves and stem)	Maceration	Methanol	Identification of polyphenols such as- epigallocatechin-catechin dimer, quinic acid, quinic acid derivative, gallic acid, catechin, chlorogenic acid, methyl -(5-caffeoyl)-quinate, epigallocatechin gallate, epicatechin, 1-*O*-sinapoyl-*β*-d-glucose, myricetin-3-*O*-(6"galloyl), galactose, methyl (3-caffeoyl)-quinate, myricetin -3-*O*-galactose (myricitrin), unknown flavonoid glycoside, apigenin (2"-hexoside)	Liquid chromatography (LC) coupled with UV detection and electrospray ionization (ESI) tandem mass spectrometry (MS/MS)-for generating chemical fingerprints HPLC-MS	[8]

From Table 2, ethanol, methanol and water are commonly used solvents for extraction of bioactive compounds from *C. monogyna* plant parts. It is noted that in a few studies, extraction was carried out with absolute solvents itself; whereas in a few, the solvent was used in different concentrations. From the above studies, the bioactive compound recovery was high in *C. monogyna* methanol (80%) extract [34].Water and ethanol extracts of flowers, leaves and fruits also had high hydrogen peroxide (H_2O_2) radical scavenging and total antioxidant activity when compared with BHA and α-tocopherol as reference antioxidants. Therefore, the results of this study revealed that the water and ethanol extracts of *C. monogyna* can be used as an easily accessible source of natural antioxidants and as a possible food supplement or in the pharmaceutical industry [87].

According to Tadic *et al*., the main classes of antioxidant bioactive compounds present in common hawthorn are oligomeric proanthocyanidins, flavonoids, triterpene acids, organic acids and sterols; a few authors also identified many individual compounds which are reported to have antioxidant activity, compounds such as chlorogenic acid, epicatechin, hyperoside, rutin, vitexin and procyanidins [30, 94, 95, 96].

As stated before, selection of solvents plays a crucial role in the extraction process because of the usage of appropriate solvent type and concentration for efficient extraction rate. However, a comparative study on different solvents applied on C. *monogyna* which aids in higher extraction rate in terms of high antioxidant activity remains a top priority. Thus, through various results and past evidence, it is clear that the use or selection of solvent type is an important criterion in any biological extraction process since the final bioactive compounds extracted are made available to mankind.

4. Techniques of isolation and identification of bioactive compounds from *C. monogyna*

4.1 Isolation of bioactive compounds isolated from C. *monogyna*

Purification and isolation of bioactive compounds from plants have gained new excellence in recent years due to the advancement of improved techniques. Advancement in these modern techniques has resulted in improving the quality of isolation, separation, purification and further analysis of the bioactive compound. Thus, by isolating the bioactive compounds, the major screening such as the bioactivity of the bioactive compound antioxidant, antibacterial, anti-inflammatory, anti-carcinogenic, cardiac-protective will be identified and potentially used to cure various diseased conditions [97, 98, 99].

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Ravikumar. Reviews in Agricultural Science, 10: 304–327, 2022
https://doi.org/10.7831/ras.10.0_304

Typically, plant material is composed of different types of tissue owing to their plant parts, hence producing quite different compounds with diverse chemical, biological and even physico-chemical characteristics. These characteristics remain a huge challenge in isolating and characterising the bioactive compounds in plants. However, the selection of plant material and their plant parts is considered as a preliminary step in any isolation technique.

Usually, chromatographic techniques are used for separation of the mixture by passing the solution in a particular medium in which the components move at different rates. Chromatographic techniques are broadly divided into four streams: liquid chromatography, gas chromatography, thin-layer chromatography and paper chromatography. Column chromatographic techniques are generally used for the isolation of bioactive compounds [100, 101].

Column chromatography and thin-layer chromatography (TLC) are performed concurrently. In order to obtain an optimum solvent system, TLC is performed prior to column chromatography experiments. Thin-layer chromatography has always been used to analyse the fractions of compounds by column chromatography. Developed instruments such as high-performance liquid chromatography (HPLC) accelerate the process of purification of the bioactive molecule [101, 102].Till date, TLC and column chromatography are mostly used due to their convenience, economy and availability in various stationary phases. Silica, alumina, cellulose and polyamide exhibit the most value for separating the phytochemicals.

Plant materials include high amounts of complex phytochemicals, which make a good separation difficult. Therefore, increasing polarity using multiple mobile phases is useful for highly valued separations. Similar to other plants, *C. monogyna* is also composed of more complex phytochemicals. Therefore, isolation of bioactive compound from *C. monogyna* remains a great challenge.

In the *C. monogyna* plant, after extraction, the crude extract of *C. monogyna* is isolated into targeted bioactive compounds. As mentioned above, chromatographic techniques such as thin-layer chromatography (TLC), column chromatography (CC), liquid chromatography (LC), liquid chromatography-mass spectrometry (LCMS), high-performance liquid chromatography with an ultraviolet detector (HPLC-UV) and high-performance liquid chromatography with mass spectroscopy (HPLC-MS) are used for the isolation of proanthocyanidins, *C*-glycosyl flavonoids, flavones, anthocyanins, phenolic acids, chlorogenic acid, hyperoside, rutin, quercetin, vitexin-2*O*-rhamnoside, epicatechin, catechin and procyanidin B2 [8, 24, 25, 26, 69] from the *C. monogyna* plant.

In previous studies, thin-layer chromatography (TLC) was employed on *C. monogyna* callus extract for the separation of proanthocyanidins and flavonoids. Total callus extracts were examined by one-dimensional thin-layer chromatography on silica gel plates [90]. Recently, liquid chromatography with mass spectroscopy (LC-MS) was used on *C. monogyna* plant extract for the isolation of proanthocyanidins [30]. Kim *et al*. (2000), used column chromatography on Sephadex LH-20 for the isolation of proanthocyanidins from *C. monogyna* plant extract for evoking the endothelium-dependent vaso-relaxation in rat aorta [13]. Liquid chromatography (LC) is an analytical chromatographic technique used for the separation of molecules that are dissolved in the solvent. LC was employed on the hawthorn plant for formulating microspheres and testing the free radical scavenging potential of the microsphere [27].

Thin-layer chromatography (TLC) is a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound. TLC is an analytical tool widely used because of its simplicity, relatively low cost, high sensitivity and speed of separation. TLC functions on the same principle as all chromatography. The major disadvantage of using

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Ravikumar. Reviews in Agricultural Science, 10: 304–327, 2022
https://doi.org/10.7831/ras.10.0_304

TLC are overlarge spots; spotting sizes of your sample should not be larger than 1–2 mm in diameter. The component spots will never be larger than or smaller than your sample origin spot.

The main disadvantage of TLC—the occurrence of overlarge spots—could cause overlapping of other component spots with similar R_f values on TLC plate. If overlapping occurs, it would prove difficult to resolve the different components; uneven use of solvents and streaking are the other disadvantages [103, 104, 105]. Thus, it is advisable to use TLC as a confirmation technique for isolation along with other chromatographic techniques.

In order to isolate every single compound from *C. monogyna* plant part extract with high antioxidant activity, column chromatography (CC) and thin-layer chromatography (TLC) are considered as the best isolation techniques. In column chromatography, the columns are usually glass or plastic (transparent) material, through which separation could be made possible by means of visualising the distinct stationary and mobile phases. In the column, the separated band may contain a single component or a mixture of few components of similar polarity in a given mixture. This can be ascertained using TLC. If it's a mixture, preparative TLC is the better technique to purify further to get the desired component. HPLC is also a good technique, especially preparative HPLC, where the purity of the eluted component is controlled using mass spectrometric detector. Hence, a combination of both TLC and CC can be a supporting tool for the isolation of bioactive compounds from *C. monogyna*. After isolation of any type of bioactive compounds, identification or structural elucidation of the isolated compound is mandatory. In section 4.2, a brief description of different structural elucidation techniques conducted on the *C. monogyna* plant are given in detail.

4.2 Identification or structural elucidation of bioactive compounds isolated from *C. monogyna*

Structure elucidation or structure identification is carried out with the help of spectroscopic techniques. Four primary spectroscopic techniques have been in use since the early 1960s: nuclear magnetic resonance (NMR), infrared (IR), ultraviolet-visible (UV-VIS) and mass spectroscopy. In *C. monogyna*, UV-visible, infrared (IR), nuclear magnetic resonance (NMR) and mass spectroscopy are technically used for the determination of structures and identification of bioactive compounds.

The basic principle of spectroscopy analysis is to pass the electromagnetic radiation through the organic molecule, which absorbs some of the radiation and emits the unabsorbed radiation. Then, by measuring the amount of absorption of electromagnetic radiation, a spectrum is produced. Depending on the spectra, the structure of the organic molecule is identified. Scientists mainly use spectra produced from either three or four regions such as ultraviolet (UV), visible, infrared and electron beam from mass spectroscopy for structure identification [99, 104].

Structural elucidation is an important phenomenon through which the chemical composition of an isolated compound can be analysed. With respect to the *C. monogyna* plant, different techniques used for structure identification are listed in Table 3.

Table 3: Structural elucidation techniques and identified bio-active compounds from *C. monogyna*

Structure elucidation technique	Identified bioactive compounds	Conditions for structural elucidation	Sources
MS	Polyphenols, proanthocyanins	NA	[29, 30]
FTIR Spectroscopy	Saccharides, uronic acids, polyphenols, proteins, flavonoids, polysaccharide	The spectra of KBr pellets (2 mg of a sample/200mg KBr) were collected in the middle region from 4000 to 400 cm^{-1}, at a resolution of 4 cm^{-1}. The degree of methylation of carboxylic functional groups (D) was determined from the ratio of the intensity of bands	[31]

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https://doi.org/10.7831/ras.10.0_304

		centred at 1738 cm^{-1} for the esterified carbonyl groups and at 1600 cm^{-1} for carboxylic anions.	
NMR	Saccharides, uronic acids, polyphenols, proteins, flavonoids, polysaccharide, anthocyanin, proanthocyanins	^{1}H and HSQC NMR are used. The spectra of samples were recorded in d_2o at 60°C on III HD 400 NMR spectrometer, using liquid N_2 cooled BB-(H-F)-D-05-Z. Chemical shifts were referenced to internal standard TSP-D_4. CD_3OD/CF_3 (98:2) mixture (v/v) on a spectrometer operating at 500 MHz for 1h- and 125 MHz for ^{13}C NMR	[29, 30, 31, 32]

From Table 3, the mass spectroscopy (MS), Fourier-transform infrared (FR-IR) spectroscopy and nuclear magnetic resonance (NMR) have been used, among which NMR techniques are commonly used for structure elucidation or identification of bioactive compound in *C. monogyna*. Mass spectroscopy is typically used for the identification of polyphenols and proanthocyanins in the *C. monogyna* plant (from Table 3). Mass spectroscopy is an analytical technique that measures the mass-to-charge (m/z) ratio of ions from a sample and results in a measurement of relative abundance. Mass spectrometry is an indispensable analytical tool in chemistry, biochemistry, pharmacy and medicine. Structure elucidation of unknowns, environmental and forensic analytics and quality control of drugs, flavours and polymers processes have a great extent on mass spectrometry [105].

Infrared spectroscopy is one of the most often used spectroscopic tools for the study of polymers. IR spectroscopy is used for the identification of saccharides, uronic acids, polyphenols, proteins, flavonoids and polysaccharide from the *C. monogyna* plant. The method is rapid and sensitive, with a great variety of sampling techniques, and the instrumentation is also inexpensive. The use of Fourier-transform infrared (FTIR) spectroscopy has been revitalised with a new generation of infrared instruments and sampling techniques which benefit structural evaluation involving configuration and conformational analysis. Initially, FTIR was used for the identification of carbohydrates; later, they were also used in the identification of phenolic compounds [106].

Nuclear magnetic resonance (NMR) allows the identification of compounds with identical masses, including those with different isotopomer distributions. NMR is the mainstay for determining structures of unknown compounds. Through the use of stable isotope labels, NMR can be used to elucidate the dynamics and mechanisms of metabolite transformations and explore the compartmentalisation of metabolic pathways. There are two different types of NMR; one-dimensional (1D) ^{1}H NMR is the most widely used NMR approach in metabolomics, and two-dimensional (2D) NMR methods offer improved approaches for unambiguous identification of metabolites in mixtures. These 2D methods include ^{1}H-^{1}H COSY (correlated spectroscopy), ^{1}H-^{1}H TOCSY (total correlation spectroscopy) and ^{1}H–^{13}C HSQC (heteronuclear single-quantum correlation) [109, 110].

Identification of bioactive compound and quantifying the compound plays a critical role since it remains as a base for clinical and evidence base studies. Comparing the three techniques—MS, FR-IR spectroscopy and NMR—mass spectrometry (MS) gives information about the molecular weight of the compound and when performed in conjunction with a combustion analysis, the relative percentages of carbon, hydrogen and oxygen present. This is quite useful in determining a molecular formula for the compound attempting to identify.

Additionally, MS also helps determine a compound with certain elements such as bromine and chlorine, based on the abundance of their isotopes. The presence of these halogens is easily detected by noticing the intensity ratios of ions differing by two atomic mass units. The major limitation of MS when compared to FTIR and NMR are that it is not useful in trying to distinguish between compounds which have the same molecular formula. Some other

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Ravikumar. Reviews in Agricultural Science, 10: 304–327, 2022
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disadvantages include that it is not able to distinguish between isomers of a compound having the same charge-to-mass (m/z) ratio [109].

Comparing FTIR spectroscopy, the basis of infrared spectroscopy is that the compound to be analysed requires a dipole moment. Therefore, the molecules with no dipole moment will not absorb infrared rays in the first place and thus cannot be analysed. FTIR spectroscopy is not suitable for minerals such as spinel group minerals (SGMs), which are supposed to hold only traces of water in their structure. Once there is iron involved in the crystal structure, it will be impossible to distinguish between the weak OH peaks and noise. The samples from crust had no manifest evidence of well-defined water detected by FTIR spectroscopy [109].

NMR is a non-destructive technique; it does not damage/destroy the sample and can use that sample to do other experiments even as standards. The major advantage of using NMR is all three phases such as solid, liquid and gaseous can be experimented on it. Whereas some instruments are limited to only the solid or liquid phase. The advantage to this is not everything can be crystallised, so being able to determine various properties of the molecule in the liquid phase is very important and considered biologically relevant. One can even conform to various dynamics, structure and more at a single-molecule level, and at different temperatures (from 77K to over 350K) [113, 114, 115, 116]. When comparing the overall techniques and its findings, only group of compounds pertaining to targeted activity have been studied and researched in *C. monogyna* plant part extracts. However, identification of pure compounds remains a major gap in *C. monogyna* plant extracts, which would aid as in influencing factor in identification of specific targeted activity of the compounds. Finally, after the identification of bioactive compound, quantification of the bioactive compound plays a vital role, since quantification determines the amount of identified compound present in the particular extract of *C. monogyna*.

5. Quantification techniques used in the C. monogyna plant

As mentioned above, quantification of a bioactive compound is an important technique since it could help in relating the activity of the bioactive compound with respect to the quantity. Table 4 provides a summary of different quantification techniques executed on the *C. monogyna* plant. This table displays the quantified bioactive compounds from *C. monogyna*, with three major techniques such as GC-MS, HPLC and UV-spectrophotometry.

Table 4: Quantification techniques and quantified bioactive compounds from *C. monogyna* plant extract

Quantification technique	Quantified bioactive compounds	Conditions for quantification of bioactive compounds	Wavelength detections	Sources
GC-MS	Monosaccharides and triterpenoids	ITQ 700 chromatograph combined with an ion trap detector, equipped with Restek Rtx-225 column (0.25 mm× 30 m) was applied. The oven temperature program was 170–180 °C (1 °C/ min), 180–235 °C (3 °C/min), and the flow rate of helium was maintained at 1 mL/min.	NA	[31]
HPLC	Epicatechin, vitexin 2″-o-rhamnoside, acetylvitexin 2″-o-rhamnoside, hyperoside, proanthocyanidins	HPLC-UV/PAD: PU-2089 plus quaternary gradient pump and MD-2010 plus multi-wavelength detector. Chromatographic separation was carried out using a Chromolith speed ROD RP_{18} end capped column (50 × 4.6 mm i.d.; 3 mm; macropore size 2 µm; mesopore size 13 nm) and a security guard	340nm	[57, 89, 92, 93, 118, 119]

	, flavonoids, polyphenols, anthocyanin, flavonoid glycosides, phenolic acids and flavonoid aglycones	H5-10C column. The mobile phase consisted of 0.4% orthophosphoric acid aqueous solution, tetrahydrofuran and isopropanol in portions of 85:10:5. Isocratic elution at a flow rate of 1.5 mL/min. HPLC-DAD: Quaternary pumps L-7100, equipped with D-7000 HSM multi-solvent delivery system autosampler L-7200. The separation was performed on cadenza CDC18 (75 mm × 4.6 mm, 5 µm) column. Mobile phase of eluent A (4.5% formic acid, v/v) and eluent B (acetonitrile). The flow rate was 1.0 mL/min. Ultrabase C18 column (5 µm; 4.6 mm × 150 mm) which was set thermostatically at 25ºC. Solvents used to analysis were acetic acid 2.5% (A), HPLC- grade acetonitrile (B), ultra-pure water (C) and acetic acid 2.5% HPLC-grade acetonitrile (90:10) (D) at a flow rate of 0.5 mL min^{-1}.	280 nm (flavonols) 320 nm (hydroxycinnamates 360 nm (flavonol glycosides)	
VU-Spectrophotometry	Flavonoids, phenols, monomeric anthocyanins and proanthocyanidins	1 ml of hawthorn extract were added 10 ml 96% (v/v) of ethanol, 0.5 ml of 33% acetic acid, 1.5 ml 10% $AlCl_3$ and 2ml 5% hexaethylenetetramine solutions. Spectrophotometric analysis was performed after 30 min. For quantitative evaluation absorption differences were compared with absorption differences of 0.025 g/50 mL rutin.	407 nm	[33, 34]

From Table 4, gas chromatography-mass spectrophotometry (GC-MS), high-performance liquid chromatography (HPLC) and ultraviolet (UV) spectrophotometry were used. Among which, GC-MS were used in quantification of monosaccharides and triterpenoids; from HPLC epicatechin, vitexin 2″-O-rhamnoside, acetylvitexin 2″-O-rhamnoside, hyperoside, proanthocyanidins, flavonoids, polyphenols, anthocyanin, flavonoid glycosides, phenolic acids and flavonoid were quantified and UV-spectrophotometry for quantification of flavonoids, phenols, monomeric anthocyanins and proanthocyanidins. Each author used specified conditions for their quantification technique based on the compounds to be quantified.

UV-visible spectroscopy can be performed for qualitative analysis and for the identification of certain classes of compounds in both pure and biological mixtures. Preferentially, UV-visible spectroscopy can be used for quantitative analysis because aromatic molecules are powerful chromophores in the UV range. Natural compounds can be determined using UV-visible spectroscopy. Moreover, spectroscopic UV-Vis techniques were found to be less selective and can give information on the composition of the total polyphenol content. UV-Vis spectroscopy was used to determine the total phenolic extract (280 nm), flavones (320 nm), phenolic acids (360 nm) and total anthocyanidins (520 nm). This technique is not time-consuming and presents reduced cost compared to other techniques [104, 117].

The major disadvantage of using GC-MS for drug confirmation testing or broad-spectrum drug screening is that GC-MS methods are not capable of directly analysing drugs that are non-volatile, polar or thermally labile.

Derivatisation is required to increase the volatility and thermal stability of these compounds. This involves derivatising one or more polar groups on a compound to a less polar group. Derivatisation can also be used to achieve increased sensitivity, selectivity or specificity for a given chromatographic separation. For drug confirmation testing and broad-spectrum drug screening, lengthy sample preparations, which include hydrolysis and derivatisation, are required prior to GC-MS analysis [120, 121]. Hence, GC-MS is typically used only for the quantification of saccharide molecules.

Speed, efficiency and accuracy are the major advantages of using the HPLC technique. It uses a pump, rather than gravity, to force liquid solvent through a solid adsorbent material, with different chemical components separating out as they move at different speeds. The process can be performed in minimum duration and it delivers a high resolution. It is accurate and highly reproducible. Despite its advantages, HPLC analysis is expensive, requiring standards and other organic HPLC-grade solvents. This is mainly because of the array of different modules, columns and mobile phases. In general, HPLC is versatile and extremely precise when it comes to identifying and quantifying chemical compounds because of the steps involved in the process, leading to high precision of HPLC down to the process being automated and therefore highly reproducible [122, 123].

With respect to UV-spectrophotometry, the main disadvantage of using UV-spectrometer is the time and need for more controlled conditions such as the absence of outside light, electronic noise or other outside contaminants that could interfere with the spectrometry reading. UV-spectrophotometry is simple to use and gives accurate results. But, external factors such as even a small bit of outside light or vibration from a small electronic device could interfere with the results. However, in contrast with the three quantification techniques employed on *C. monogyna*, HPLC technique is considered as the best quantification technique with added advantages; to note, HPLC has gained more importance in recent times.

Extraction, isolation, identification and quantification are important fundamental techniques which could help in furthermore emerging techniques such as microencapsulation, through which the bioactive compounds could be made available to mankind. Thus, the four major analytical techniques such as extraction, isolation, identification and quantification go conjoinedly with one another. In a series of analytical procedures, it is necessary to follow appropriate protocols as per the technique used. Any dysfunction in selected technique or in protocol could affect the ensuing analytical procedures. However, this review helps in portraying the advantages and disadvantages of each analytical technique applied on the *C. monogyna* plant, serving the reader to identify the best technique and its limitations.

6. Future aspects of the *C. monogyna* plant

Thus, *C. monogyna*, a promising shrub, could be used as an alternate source of standardised natural herb formulation with negligible side-effects. Also, the European Pharmacopeia has stated *C. monogyna* as a safe herb for human consumption. Hence, by identifying *C. monogyna* plant part and solvent type, isolating single pure antioxidant-rich bioactive compound and finally, microencapsulating could serve as an alternate for available commercial drugs with standardised natural herb formulation, which would in turn help in decline of mortality rate caused by CVD.

There are different types of cardiovascular diseases, which include coronary heart disease, cerebrovascular disease, hypertension, peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure. World Heart Federation stated that the world's most common cause of death is due to cardiovascular diseases. Over 17 million global deaths are due to CVDs; that is, 31% of the world population. It is also predicted that it would increase to over 23 million by 2030; heart disease and stroke cause a third of all deaths in women worldwide [122,

126]. Based on the mortality rate in Malaysia 2015, including all genders and all ages, 35% of deaths are due to cardiovascular diseases [124].

In contrast with drug prevalence, consumption of synthetic drugs results in free radical oxidative stress, which in turn results in damage to the heart muscle [125]. Drugs such as anticoagulants, antiplatelet agents, DAPT (dual antiplatelet therapy), ACE inhibitors (angiotensin-converting enzyme), angiotensin II receptor blockers, angiotensin-receptor neprilysin inhibitors, beta-blockers, calcium channel blockers, cholesterol-lowering medications, diuretics and vasodilators are the common medications prescribed for cardio patients [126]. Prolonged intake of such drugs results in minor complications to severe complications such as heart failure, paralysis and stroke, which again ensues in cardio disturbances. However, to overcome this vicious cycle, there is a need for a substitute for synthetic or commercial drugs with standardised natural plant extract formulation.

In order to produce a standardised natural extract, there is a lack of system in incorporating a suitable alternate by means of plant source with low or no toxic effect on individuals. The lack of proper extraction technique and lack of knowledge in selection of appropriate solvent for extraction, selection of plant material and type of bioactive compounds to be extracted, results in the need for standardised techniques through which antioxidant-rich extract could be extracted.

To summarise, the main motivational factors to take up this review article is an increase in the number of cardiac-related deaths globally due to various reasons and causes. One of the elevating factors is that increase in the use of synthetic drugs for a prolonged period of time results in free radical oxidative stress, which in turn, ends up in damage to the heart muscle and finally in congestive heart failure. An alternative to avoid such a condition is the use of standardised natural plant extract as a source of medicine with negligible toxic effects.

7. Conclusion

Among the *Crataegus* species, *C. monogyna* extracts are reported to have positive effects on reducing the symptoms of congestive heart failure; it is necessary to utilise the shrub efficiently, which has grabbed the attention of many researchers. Apart from the various techniques employed on the plant, isolation, identification and quantification of plant extract responsible for high antioxidant activity remains a major gap. Since *C. monogyna* is considered as a diversified plant and has established its role in treating various diseased conditions, the role of a single pure compound responsible for high antioxidant activity is yet to be analysed.

The science of chemistry and pharmacological activity of *C. monogyna* extract has a wide opportunity in future researches. The current review provides a detailed explanation of analytical techniques employed on the *C. monogyna* plant, directing future researchers in the identification and analysis of appropriate techniques, with parameters to be considered along with highlighted gaps.

Acknowledgement

The authors would like to acknowledge Ministry of Higher Education (MOHE), Malaysia for providing financial support under the Fundamental Research Grant Scheme, FRGS/1/2022/TK05/TAYLOR/02/1.

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