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Research Article

Phytochemical Screening, Antioxidant and Anti-Arthritis Potential of Decoction Extract from *Caulerpa lentillifera*

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ABSTRACT

Caulerpa lentillifera also known as sea grape, is a green seaweed in the Caulerpaceae family known for its nutritional benefits and medicinal properties. This study aimed to examine the phytochemical compounds, total phenolic content, total flavonoid content, and antioxidant and anti-arthritis properties of sea grape decoction extract. The total phenolic content was measured using the Folin-Ciocalteu method, and the total flavonoid content was determined with the aluminum chloride colorimetric method. Antioxidant activities were evaluated using two methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis-(3-ethylbenzothia-zoline-6-sulfonate) (ABTS). The anti-arthritis activities were assessed using bovine-serum protein. The study identified that the extract contained flavonoids, saponin, terpenoids and coumarins, with the total phenolic content of 343.19 ± 9.86 µg gallic acid equivalents/ mg dry extract and the total flavonoid content of 268.48 ± 16.57 µg quercetin equivalents/ mg dry extract. The extract showed antioxidant activity with an IC_{50} value of 309.05±5.91 µg/ml using the DPPH method and 572.33±3.47 µg/ml using the ABTS method, respectively. Additionally, it exhibited anti-arthritis properties with an IC_{50} value of 54.60±12.71 µg/ml, which was less than 1.69 times the IC_{50} value of diclofenac sodium. A strong positive correlation was found between the antioxidant and anti-arthritis activities measured by the ABTS method, with an R^2 value of 0.9476. Therefore, this study provides initial evidence supporting the antioxidant and anti-arthritis activities of sea grape decoction extract. The extract shows potential for further development as a product for treating arthritis.

Keywords: *Caulerpa lentillifera*, Phytochemical components, Antioxidant activity, Anti-arthritis activity.

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INTRODUCTION

The human body possesses an intricate mechanism to counteract free radicals, comprising both natural enzymes and non-enzyme components. This helps to reduce the dangers of free radicals and substances that cause free radicals.[1] Excessive free radicals in the body, which derive from reactive oxygen species (ROS) produced during cellular metabolism, can lead to the development of various diseases.[2] For example, cancer, diabetes, cardiovascular diseases, chronic inflammation, arthritis, and rheumatoid arthritis.[3] Arthritis is a common problem among the elderly and affects a significant portion of the global population, with one in five individuals experiencing this condition, leading to weakened bodily strength.[4] Treating arthritis and inflammation typically requires medications for example non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying antirheumatic drugs (DMARDs) and corticosteroids. The side effects of NSAIDs may include irritation of the gastrointestinal tract and gastric ulcers. Prolonged use, especially in combination, can lead to decreased liver and kidney function.[5] According to a report from the World Health Organization, approximately 80 percent of the global population uses herbal medicine for managing illnesses and promoting well-being.[6] Therefore, researchers are interested in herbal plants because modern medications may have side effects on health and be expensive.[7] In Thai Traditional medicine, the common method for using herbal plants was through decoction.[8] Decoction extraction involves simmering the plant in a sufficient amount of water for a period ranging from 30 minutes to 2 hours, followed by filtering out the residue.[9] Research comparing maceration and aqueous techniques revealed that decoction can improve the solubility of alcohol, flavonoids, polyphenols, and tannins[10]. Therefore, it is still a challenge to obtain antioxidant and anti-arthritis agents from plants.

Caulerpa lentillifera (sea grape), belonging to the Caulerpaceae family, is highly favored for its vibrant green color, tender, and juicy texture, commonly enjoyed fresh as a vegetable or in salads. It is grown in ponds and open lagoons throughout the Philippines. Many farmers in Thailand, grow sea grapes in ponds for water treatment[11]. Sea grapes are rich in essential amino acids, vital minerals like Zn, Fe, Co, Cr, Mo, Ni, Se, and V, and polyunsaturated fatty acids (PUFAs). They are frequently consumed as food or extracts to enhance health.[12] Details regarding the pharmacological characteristics of sea grapes includes anti-inflammatory, immune-enhancing, blood sugar-lowering, antiplatelet aggregation, antibacterial, fever-reducing, anti-cancer, antioxidant, heart-protective, and chelating agents.[13] The chemical constituents found in water-based extracts of sea grapes include sterols, phenols, and saponins.[14] A study on the total phenolic content in sea grape aqueous extract found that it contained 2.04 ± 0.36 mg GAE/g extract. The total amount of flavonoid was equivalent to 1.17 ± 0.03 mg QE/g extract. The aqueous extract of sea grape demonstrated DPPH radical scavenging activity, with an EC_{50} value of 81.55 ± 4.22 mg/ml.[15] The study found that seaweeds of the phylum Phaeophyta exhibited anti-arthritis properties of up to 60 percent. Nonetheless, a single clinical trial examined

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the Maritech® seaweed extract formula, comprising extracts from *Fucus vesiculosis*, *Macrocystis pyrifera*, and *Laminaria japonica*. The findings indicated that this formula could alleviate osteoarthritis symptoms in a dosage-dependent manner in patients with osteoarthritis.[16]

Based on the literature review, there have been no findings on the anti-arthritis effects of sea grape decoction extract. Therefore, this study aimed to identify the phytochemical compounds, quantify the total phenolic, flavonoids contents, assess antioxidant activity using DPPH and ABTS assays. Moreover, investigate anti-arthritis properties, and explore the relationship between antioxidant and anti-arthritis effects of sea grape decoction extract.

MATERIAL AND METHODS

Material

The reagents and chemicals used for phytochemical screening and pharmacological activity, including ferric chloride solution, glacial acetic acid, sulfuric acid, sodium hydroxide, ammonia solution, and ascorbic acid, were procured from QReC (New Zealand). Hydrochloric acid, chloroform and absolute ethanol were purchased from RCI Labscan (Thailand). Dragendorff's reagent was purchased from Sigma Aldrich (United States). Folin–ciocalteu phenol reagent, aluminum chloride, gallic acid and phosphate buffer saline (PBS) were purchased from SRI chemical (India). Sodium carbonate and aluminum chloride were purchased from KemAus (Australia). Quercetin and Butylated Hydroxytoluene was purchased from MySkinRecipes (Thailand). ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH(2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma-Aldrich (USA). Albumin bovine and phosphate buffer were purchased from Sisco Research Laboratories (India).

Plant material collection

The sea grapes were harvested from a farm in Laem Phak Bia sub-district, Ban Laem district, Phetchaburi province (13°03'75.7" N 100°07'98.8" E), in March 2023. Afterward, the sea grapes underwent cleaning, cutting, drying, and grinding into powder.

Preparation of the decoction extract

Following the decoction method in Thai traditional medicine[17], 100 g of each of sea grape powders were boiled in 1,500 ml of distilled water until two hours. The decoction extract was filtered using both a muslin cloth and Whatman No. 01 filter papers. The combined filtrates were then freeze-dried (CHRIST, Germany) at -60 degrees celsius for a duration of 3 days. Once dried, store the extract in amber glass bottles and preserve the herbs in a refrigerator at 4 degrees Celsius. Determine the percentage of the extract using the following equation (1):

$$\text{Yield (\%)} = \frac{\text{Weight of dried crude extract (g)}}{\text{Weight of dried plant}} \times 100 \quad (1)$$

Phytochemical screening

Screening of the sea grape decoction extracts for tannin, flavonoids, alkaloids, saponin, terpenoids, phlobatannin, coumarins and anthraquinones constituents were carried out using modified techniques.[18,19]

Tannins Test

About 0.2 g of powdered sample, dissolve it in 5 ml of distilled water, and boil for 2 minutes. After filtering the solution, add 3 drops of 1% $FeCl_3$ solution. A greenish-black or dark navy blue color indicates the presence of tannin.

Flavonoids Test

To 0.2 g of extract was added in 3 ml of 50% methanol solution. Then, three small pieces of magnesium ribbon were introduced into the filtered solution. Then, a small piece of 3 pieces of magnesium ribbon, was added to the filtered solution. The mixture was brought to a boil, and left to cool, and 1-3 drops of 95% HCl were added. If the solution turns yellow, orange, or red, it indicates the presence of flavonoids.

Alkaloids Test

To 0.2 g of extract was added in 15 ml of 2% CH_3OH, then heat for 3 minutes. After filtration, add a few drops of Dragendorff's reagent. The formation of reddish-orange precipitates indicates the presence of alkaloids.

Saponins Test

0.5 grams of extract was added in a test tube, then 5.0 ml of distilled water were added and shaken vigorously. The persistent froth that lasted for about 15 minutes indicated the presence of saponins.

Terpenoids Test

To 0.5 g of extract, 2 ml of $CHCl_3$ was added. Then, 3 ml of concentrated H_2SO_4 was carefully layered on top. The appearance of a reddish-brown color at the interface indicates the presence of terpenoids.

Phlobatannins Test

To 0.2 g of extract, add 10 ml of CH_3OH and 1 ml of H_2SO_4. A red precipitate signifies the presence of phlobatannins.

Coumarins Test

Dissolve 0.2 g of extract in 1 ml of 50% C_2H_5OH, then add 1 ml of 6M NaOH. The appearance of a yellow color indicates the presence of coumarins.

Anthraquinones Test

0.2 grams of extract was boiled with 1 ml of 10% H_2SO_4 for a few minutes in a water bath. The reaction mixture was then filtered and allowed to cool, added 0.5 ml of 10%NH_3. The mixture was heated, and the formation of a rose-pink color indicates the presence of anthraquinone.

Total Phenolic Content

The total phenolic content was measured using the Folin-Ciocalteu method.[20] The decoction extract of sea grape and gallic acid (3.9–2,500 µg/ml) were placed in a 96-well plate (50 µl per well). Then, 100 µl of Folin–Ciocalteau reagent (10% v/v) was added to each well, mixed thoroughly, and incubated at room temperature for 4 minutes. Following this, 50 µl of Na_2CO_3 solution (10% w/v) was added, and the mixture was incubated in the dark for 60 minutes at room temperature. Absorbance was then measured at 765 nm using a UV-Vis spectrophotometer. The total phenolic contents were calculated using calibration curves for gallic acid.

Total Flavonoid Content

The total flavonoid content was determined with the aluminum chloride colorimetric method.[21] Quercetin and decoction sea grape extract solutions at concentrations of 3.9–1,000 µg/ml (100 µl/well) were added to 96-well plates along with $NaNO_2$ (5%, 30 µl). After incubating for 5 minutes, $AlCl_3$ (2% w/v, 50 µl) was added and incubated for another 6 minutes, followed by a 10-minute incubation with NaOH (1N, 50 µl). The absorbance of the mixture was measured at 510 nm using a UV–Vis spectrophotometer. The total flavonoid contents were calculated using calibration curves for quercetin.

Antioxidant activity using DPPH method

The DPPH free radical scavenging capacity of the decoction sea grape extract was modified from a previous study[22] and compared with ascorbic acid, butylated hydroxytoluene (BHT). For this evaluation: ascorbic acid, and decoction sea grape extract were dissolved in deionized water, while BHT was dissolved in 95% ethanol, at concentrations ranging from 3.9 to 2.000 µg/ml. The DPPH solution was prepared in ethanol at a concentration of 500 µM. A mixture of 100 µl of ascorbic acid, BHT or sea grape decoction extract was combined with 100 µl of DPPH solution. The mixtures were incubated at room temperature in the dark for 30 minutes. Absorbance was measured at 517 nm using a UV–Vis spectrophotometer. The radical scavenging activity percentage was calculated using Equation (2).

$$\text{DPPH Free radical scavenging}(\%) = \frac{A-B}{A} \times 100 \quad (2)$$

where A is the absorbance of the reaction with control and B is the absorbance of the reaction with the extract.

Antioxidant activity using ABTS method

The ABTS scavenging assay was adapted from a previous study.[22] A mixture of 20 µl of ascorbic acid and sea grape decoction extract were diluted in deionized water, while BHT was diluted in 95% ethanol, with concentrations ranging from 3.9 to 2,000 µg/ml. This mixture was combined with 180 µl of ABTS solution. After that, incubated at room temperature in the dark for 15 minutes. Absorbance was measured at a wavelength of 734 nm using a UV–Vis spectrophotometer. The percentage of radical scavenging activity was calculated using equation (3).

$$\text{ABTS Free radical scavenging}(\%) = \frac{A-B}{A} \times 100 \quad (3)$$

where A is the absorbance of the reaction with control and B is the absorbance of the reaction with the extract.

Anti-arthritic activity

The use of extracts to inhibit protein denaturation *in vitro* using bovine serum albumin (BSA) methods[23]. The BSA solution was prepared by dissolving 5 g of BSA in 100 ml of distilled water. Then, dissolve 10 mg of the extract with 10 ml of distilled water to prepare a concentration of 1 mg/ml. The sea grape decoction extract, diclofenac sodium solution was prepared by diluting to concentrations of 31.75, 62.5, 125, 250, 500, and 1000 µg/ml. The mixture was prepared by adding 2 ml of the test solution to 2.8 ml of PBS and 0.2 ml of BSA solution. All the mixtures in the test tubes were incubated at 37 °C for approximately 30 minutes, after that, incubation at 57 °C for 5 minutes. The absorbance was measured at 660 nm.

Statistical Analysis

Data were expressed as mean ± SD of experiments performed in triplicate. The IC50 values were calculated from log dose-response curves using GraphPad Prism software version 10. Pearson correlation statistics were used to analyze the relationship between the percentage of antioxidant activity and the percentage of anti-arthritis of extract.

RESULTS

Phytochemical Screening

The decoction extract of sea grape contains a yield percentage of 16.15±2.82%. The results of the extract contain flavonoids, saponins, terpenoids, and coumarins, as shown in Table 1.

Total phenolic and total flavonoid contents

The total phenolic content (TPC) of the sea grape decoction extract was quantified using gallic acid equivalents. The standard curve equation was y = 0.3986x+0.1729, r^2=0.998 (Figure 1A). The average of total phenolic contents was 343.19±9.86 µg GAE/mg extract. The total flavonoid content (TFC) was expressed in terms of quercetin equivalents. The standard curve equation was y = 3.017x-0.0031, r^2=1 (Figure 1B). The average of total flavonoid contents was 268.48 ± 16.57 µg QE/mg extract.

Anti-oxidant activity of *C. lentillifera* decoction extract

DPPH Free Radical Scavenging Activity

The decoction extract from sea grapes extract showed DPPH free radical scavenging activity with the IC_{50} value of 309.05±5.91µg/ml. The IC_{50} values of ascorbic acid, BHT were 14.31 ± 1.15, 32.40 ± 0.21µg/ml, respectively. These findings showed that the capacity of antioxidants in scavenging the DPPH radical followed this sequence: ascorbic acid > BHT > *C. lentillifera* decoction extract (Table 2).

Table 1: Phytochemical screening of decoction extract from *Caulerpa lentillifera*.

Phytochemical tests	Caulerpa lentillifera extract
Tannin	-
Flavonoid	+
Alkaloid	-
Saponin	+
Terpenoids	+
Phlobatannin	-
Coumarin	+
Anthraquinone	-
Steroids	-

Note: + = Presence, - = Absence

Table 2: Antioxidant activity of the decoction extract from *Caulerpa lentillifera*.

Sample	IC_{50} of DPPH (µg /ml)	IC_{50} of ABTS (µg /ml)
Ascorbic acid	14.31±1.15	12.85±0.81
Butylated hydroxytoluene	32.40±0.21	20.81±0.44
Caulerpa lentillifera	309.05±5.91	572.33±3.47

Note: Data represent mean ± SD from 3 experiments

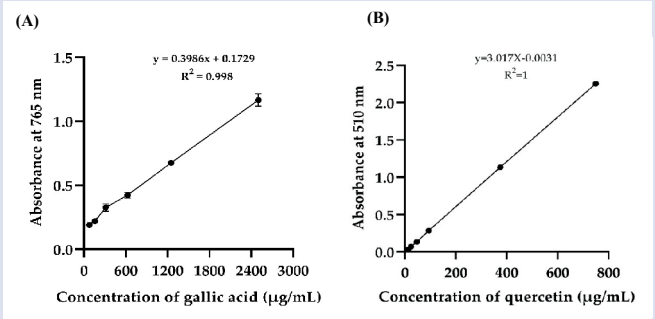

Figure 1: (A) Calibration curve of gallic acid and (B) Calibration curve of quercetin.

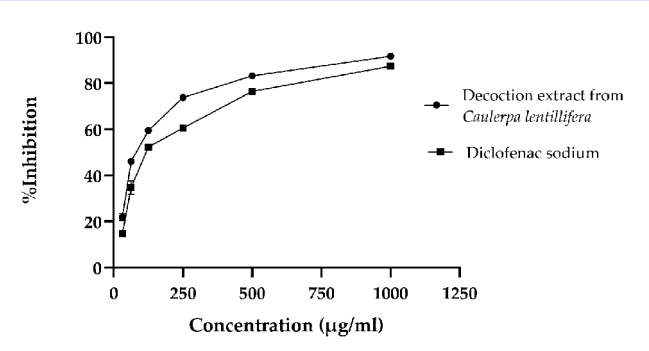

Figure 2: Percent of BSA denaturation inhibition of decoction extract from *Caulerpa lentillifera* and diclofenac sodium.

Table 3: Percent of BSA denaturation inhibition of decoction extract from *Caulerpa lentillifera* and diclofenac sodium.

Concentration (µg/ml)	% inhibition of decoction extract from Caulerpa lentillifera	% inhibition of diclofenac sodium
31.25	21.87±1.46	14.66±0.66
62.5	46.06±1.05	34.73±2.92
125	59.48±0.57	52.29±0.14
250	73.76±0.58	60.50±0.60
500	83.21±0.92	76.43±0.39
1,000	91.67±0.69	87.40±0.36
IC_{50}	54.6±12.71	92.51±8.88

Note: Data represent mean ± SD from 3 experiments

Table 4: Pearson correlation coefficients of antioxidants activity and anti-arthritis activity of decoction extract from *Caulerpa lentillifera*.

Anti-oxidant activity	The Pearson's Correlation (R^2) anti-arthritis activity
DPPH	0.7370
ABTS	0.9476

ABTS Free Radical Scavenging Activity

The decoction extract from sea grapes extract showed ABTS free radical scavenging activity with the IC_{50} value of 572.33±3.47µg/ml. The IC_{50} values of ascorbic acid, BHT were 12.85 ± 0.81, 20.81 ± 0.44 µg/ml, respectively. These results reveal that the ability of antioxidants to scavenge the ABTS radical was in the following order: ascorbic acid > BHT > *C. lentillifera* decoction extract (Table 2).

Anti-arthritic activity

The anti-arthritic activity of the decoction extract from sea grapes was tested by inhibiting bovine serum albumin and compared to diclofenac sodium. It was found that increasing the concentrations of the extract and drug enhanced the anti-arthritic activity, as shown in Figure 2. When calculating the IC_{50} values for inhibiting arthritis, the decoction extract from sea grapes exhibited the highest anti-arthritic activity with an IC_{50} value of 54.6±12.71 µg/ml, whereas diclofenac sodium had an IC_{50} value of 92.51±8.88 µg/ml (Table 3). The decoction extract of sea grapes demonstrates an anti-arthritis effect that is 1.6 times more effective than the diclofenac sodium.

Pearson correlation of antioxidants activity with anti-arthritic activity of the decoction extract from sea grapes

The study analyzed the relationship between the antioxidant activity, as measured by DPPH and ABTS methods, and the anti-arthritis effect of the decoction sea grape extract at a concentration of 1,000 µg/ml. The results showed that the antioxidant activity measured by the ABTS method had a strong correlation (R^2=0.9476), while the DPPH method had a moderate correlation (R^2=0.7370) with the anti-osteoarthritis effect (Table 4).

DISCUSSION

This study aimed to investigate the phytochemical compounds, as well as the antioxidant and anti-arthritic activities of sea grape decoction extracts. The extract contains a yield percentage of 16.15±2.82%. Nurjanah et.al. (2019) report that the yield of boiled sea grape extract was 6.36 %. Bioactive compounds in sea grape are mainly soluble in polar solvents.[14] The yield amount affects the solubility properties of these bioactive compounds. The results showed that the decoction extract exhibited the flavonoids, saponin, terpenoids and coumarin. Previous studies also reported that the sea grape fresh and boiled extract contains steroids, fenol hidroquinon and saponin, with triterpenoid being present only in boiled extract.[14] Pires-Cavalcante et al.(2011) demonstrated that seasonal variations can changes the levels of constituents in Caulerpa species.[24] The average of TPC was 343.19±9.86 µg GAE/mg extract. Previous study reported that TPC of sea grape boiled extract in Indonesia was 16.8 + 1.53 mg GAE/g extract.[14] A study report of sea grape water extracted cultivated in Malaysia found that it contains a TPC of 2.04±0.36 mg GAE/g.[15] Recently, a study found that ethanol extracts of sea grape from Phetchaburi province have a TPC of 44.69 ± 1.91 mg GAE/g.[25] This study supports other research showing that the decoction method results in higher phenolic compound levels than the maceration[26] and infusion method.[27] The average of TFC was 268.48 ± 16.57 µg QE/mg extract. Previous study reported that TFC of sea grape water extract in Malaysia was 1.17 + 0.03 mg GAE/g extract.[15] The results revealed that the decoction extract of sea grape had a high flavonoid content. Our total flavonoid contents are in accordance with the results described by Uslu, N. The decoction method usually produced greater amounts of total flavonoids with these levels rising as the brewing time was prolonged.[28]

We evaluated the antioxidant potential of the extracts using the DPPH and ABTS assays. Both assays function through the same antioxidant action mechanism, specifically electron transfer. However, DPPH radicals are more appropriate for lipophilic antioxidants, whereas ABTS+ radicals can interact with both hydrophilic and lipophilic antioxidants[29]. The decoction extract from sea grape demonstrated DPPH free radical scavenging activity, with an IC_{50} value of 309.05 ± 5.91 µg/ml. Yap et al.(2019) report that the sea grape extract by water had the IC_{50} value of 81.55 ± 4.22 mg/ml.[15] Osotprasit et al. (2021) showed that EC_{50} values of sea grape extracted by water had the EC_{50} values more than 5,000 µg/ml.[30] A study reported that ethanol extract of sea grape demonstrated DPPH radical scavenging activity with an EC_{50} value of 14.56 ± 0.32 mg/ml.[25] The findings of this study accordance with previous reports that identified significant antioxidant activity in the decoctions of plants using the DPPH method.[31] Additionally, González-Palma et al.(2016) reported that boiling the extract selectively increased its DPPH radical scavenging potential compared to the fraction prepared at room temperature.[32] The decoction extract of sea grape exhibited ABTS free radical scavenging activity, with an IC_{50} value of 572.33 ± 3.47 µg/ml. The study results revealed the ethanolic extract from sea grapes demonstrated antioxidant properties inhibit ABTS radicals, with an EC_{50} value of 7.23 ± 0.51 mg/ml.[25] The high-molecular weight polysaccharide (galactose, mannose and xylose) extracted from sea grapes showed antioxidant activity, with an IC_{50} value of 10.79 mg/ml for ABTS.[33] The variation in results between this study and other literature reviews is likely attributed to the polarity of the solvent used. The extraction of phenolic and antioxidant compounds with polar solvents influences the quantity of the extract due to the interactions (such as hydrogen bonding) between the polar sites of the antioxidant compounds and solvent, which are more effective compared to interactions with non-polar solvents.[34]

Arthritis or protein denaturation happens when proteins undergo changes in their tertiary and secondary structures due to external factors.[35] The decoction extract of sea grapes exhibited anti-arthritis activity, with a IC_{50} value of 54.60±12.71 µg/ml. This study indicate that the extract inhibits the denaturation of bovine albumin in dose dependent manner. This research is the first investigation into the potential of sea grape extract for treating arthritis. Looking to similar studies in literature, several bioactive phytochemical compounds present in medicinal herbs have shown potential efficacy against arthritis including: flavonols (quercetin), coumarins (scoparone and scopoletin), terpenes (limonene), and steroidal saponin (seiboldogenin).[36] These compounds are found in the sea grape extract obtained through decoction and maceration with ethanol.[25] Koodkaew, et al. (2024) reported the phenolic compounds found in sea grape ethanolic extract using LC-QTOF MS/MS analysis are cinnamic acid and cinnamaldehyde. The flavonoids identified include (-)-epigallocatechin, fisetin, galangin, kaempferol, and quercetagetin. The coumarins discovered are 6-methylcoumarin, 7-hydroxycoumarin, coumarin, esculetin, and hyme-cromone. A recent report indicates that cinnamic acid plays a role in TLR4/PI3K/AKT/NFkB signaling to suppress inflammation activation in rheumatoid arthritis and arthritis. [37]

The correlation between the radical scavenging activity (measured using DPPH and ABTS assays) and the anti-arthritis effects of sea grape decoction extract at a concentration of 1000 µg/ml are classified as weak (R = 0.00 to +0.49), moderate (R = 0.50 to 0.79), or strong (R = 0.80 to 1.00).[38] Analysis of the correlation between antioxidant activity and anti-arthritis effects revealed that the antioxidant activity of sea grape decoction extract, measured by the ABTS method, is strong positively correlated with its anti-arthritis effects. This correlation indicates the high antioxidant activity using the ABTS method in the decoction sea grape extract corresponds to a stronger anti-arthritis effect. The R value for the relationship between DPPH activity and anti-arthritis effects was classified as having a moderate positive correlation. Elisha et al. (2016) reported that the extracts' ability to scavenge free radicals may be linked to their potential for enhancing the immune system in the treatment of arthritis.[3] Cinnamaldehyde substants demonstrated potential in decreasing ROS formation and pro-inflammatory cytokines in arthritic rats.[39] The data indicates that sea grape extracted using a traditional decoction method contains significant compounds like flavonoids, saponins, terpenoids, and coumarin. These components contribute to the antioxidant properties, which serves as a mechanism for managing arthritis.

CONCLUSION

The study showed that sea grapes extracted using decoction contain phenolic and flavonoid compounds known for their antioxidant properties, confirmed through DPPH and ABTS assays. These antioxidants are shown to be more effective inhibiting protein denudation compared to standard drug. The study emphasizes the reliable extraction method of important compounds from sea grape *via* decoction, which could potentially lead to the development of dietary supplements designed to alleviate arthritis symptoms. However, further investigations are necessary to qualitatively identify the chemical constituents in the extract and to formulate herbal supplements or preparations for treating arthritis.

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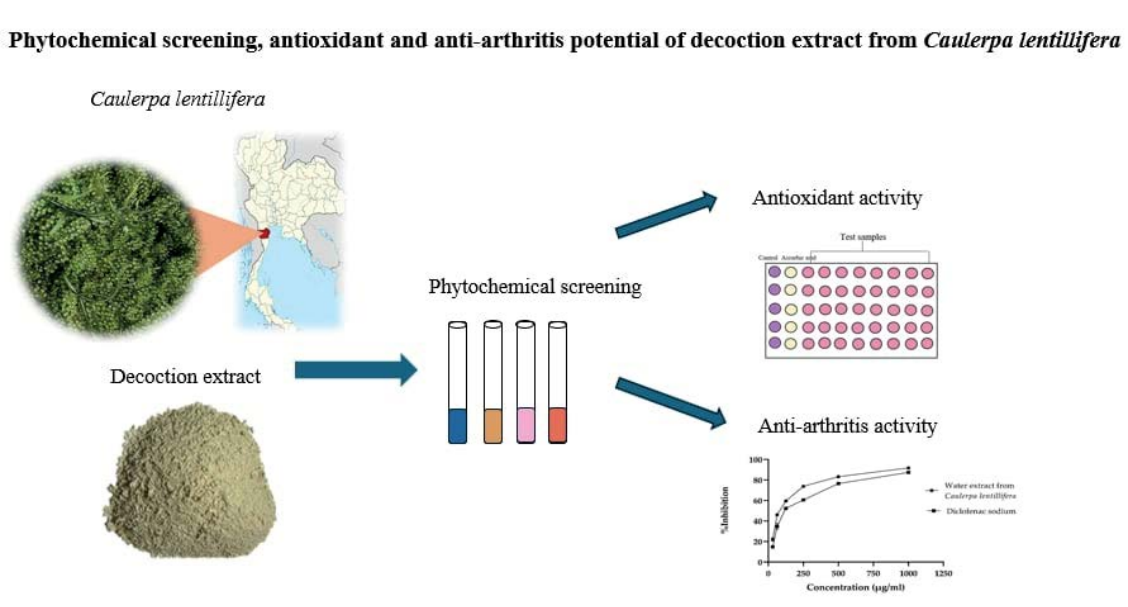
GRAPHICAL ABSTRACT



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