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INVITRO PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF *ARGEMONE MEXICANA*

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ABSTRACT

This research aimed to investigate the phytochemical composition and antioxidant potential of *Argemone Mexicana*, a medicinal plant with diverse traditional uses. The study employed in vitro methods to analyze the phytochemical constituents and assess the antioxidant activity of various extracts from *Argemone Mexicana*.The phytochemical analysis revealed the presence of bioactive compounds such as alkaloids, flavonoids, phenols, tannins, and saponins. These compounds are known for their therapeutic properties and may contribute to the plant's medicinal efficacy. Additionally, the study assessed the antioxidant activity of *Argemone Mexicana* extracts using established assays, including DPPH radical scavenging and ferric reducing antioxidant power (FRAP). The results demonstrated significant antioxidant potential, suggesting that *Argemone Mexicana* possesses compounds capable of neutralizing free radicals and preventing oxidative stress.Furthermore, the research explored the correlation between phytochemical content and antioxidant activity, providing valuable insights into the potential health benefits of *Argemone Mexicana*. The findings highlight the plant's promising role as a natural source of antioxidants, supporting its traditional use in folk medicine and emphasizing its potential applications in

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pharmaceutical and nutraceutical industries. This research contributes to the understanding of the pharmacological properties of *Argemone Mexicana* and underscores its significance in the development of novel therapeutic agents.

Keywords: *Argemone Mexicana*, antioxidant, phytochemicals, DPPH radicals, Health, Well-being, Diseases, Medical, Healthy lives

INTRODUCTION

The exploration of natural sources for bioactive compounds has gained significant attention in the field of medicinal and pharmaceutical research. Plants, being a rich repository of diverse chemical compounds, have been a perennial source of therapeutic agents. Among these, *Argemone mexicana*, commonly known as Mexican poppy or prickly poppy, is a plant that has been traditionally recognized for its pharmacological potential(1). This research endeavors to delve into the phytochemical constituents and antioxidant activity of *Argemone mexicana*, shedding light on its potential health benefits.*Argemone mexicana* is a member of the Papaveraceae family and is widely distributed in various tropical and subtropical regions across the globe. Recognizable by its vibrant yellow flowers and spiny leaves, this plant has been used in traditional medicine by diverse cultures(2,3). In folk medicine, Argemone mexicana has been employed for the treatment of various ailments, including skin disorders, respiratory issues, and inflammatory conditions. The therapeutic potential of this plant has sparked scientific interest, prompting investigations into its phytochemical composition and biological activities.Phytochemicals, the naturally occurring chemical compounds in plants, are known for their diverse biological activities(4).

Alkaloids, flavonoids, tannins, saponins, and terpenoids are among the many classes of phytochemicals that contribute to the medicinal properties of plants(5–7). These compounds often act synergistically, providing a broader spectrum of therapeutic effects(8). *Argemone mexicana*, like many other medicinal plants, is believed to harbor a rich array of phytochemicals that may account for its traditional uses.Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify them, has been implicated in various diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions. Antioxidants play a crucial role in neutralizing ROS, thus mitigating the damaging effects of oxidative stress(2). Plants are well-known for their antioxidant properties, and studying the antioxidant potential of *Argemone mexicana* could unveil novel sources of natural antioxidants with therapeutic implications.Despite the traditional use of *Argemone mexicana* in folk medicine, there is a paucity of comprehensive scientific studies elucidating its phytochemical composition and antioxidant potential(9). Investigating these aspects is not only pertinent for understanding the plant's medicinal value but also for identifying potential bioactive compounds that could be harnessed for the development of new therapeutic agents.

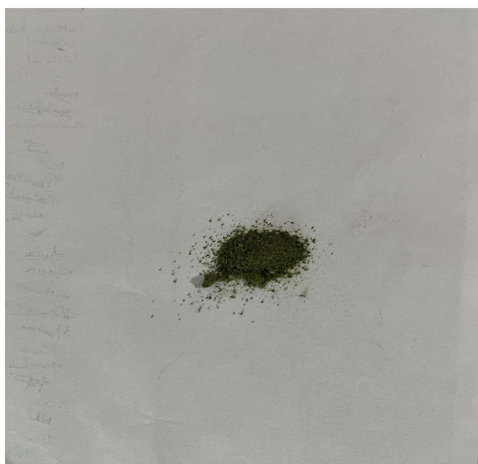
This research holds significance in several aspects. Firstly, it contributes to the scientific understanding of the phytochemical profile of *Argemone mexicana*, potentially uncovering novel bioactive compounds. Secondly, the assessment of antioxidant activity can provide insights into the plant's potential in combating oxidative stress-related diseases. Finally, the findings may lay the groundwork for the development of pharmacologically active compounds from this plant, contributing to the ongoing search for new and effective therapeutic agents(5,6).The exploration of *Argemone mexicana*'s phytochemical composition and antioxidant activity represents a crucial step in unraveling the therapeutic potential of this traditional medicinal plant. This research not only contributes to the body of knowledge on natural products but also paves the way for future studies aimed at harnessing the therapeutic benefits of *Argemone mexicana* in modern medicine.

MATERIALS AND METHODS

Preparation of Methanolic Extracts

Approximately 400 g of each of the powdered leaves was soaked in a liter of analytical grade methanol in a 2-liter capacity conical flask (Figure 1). The flasks containing each plant material were shaken regularly, corked, and left to stand for 48 hours at room temperature. In each case, the menstruum was separated by filtration through Whatman filter paper No. 1. The filtrates were then concentrated using a rotary evaporator at 50°C and later in a hot-air oven at 35°C to dry completely. The concentrates were put in airtight containers and stored at 4°C awaiting use in in vitro bioassay.

Figure 1: Powdered plant product used in our study



Qualitative Phytochemical Screening

Qualitative tests for various phytochemicals present in the methanolic leaf extracts were carried out using standard phytochemical screening procedures. Visual examination of the appearance of color or frothing was used as an indicator for the presence or absence of a given phytochemical group.

Test for Saponins

About 2 g of each of the studied plant extracts was weighed and dissolved in 5 ml of distilled water. Thereafter, aliquots of 2 ml were taken from each plant extract solution, stirred for 30 seconds, and briskly agitated. The setups were then allowed to settle for 15 minutes. The presence of frothing, which persists for over 15 minutes, is an indication of the presence of saponins in the tested sample .

Test for Alkaloids

About 2 g of each of the studied plant extracts was added to 10 ml of 0.1 M hydrochloric acid, warmed in a waterbath (50°C) for 5 minutes, and filtered through Whatman filter paper No. 1. After cooling, 3 drops of Dragendorff's reagent were added and mixed. The appearance of a reddish-brown color is a positive indication for the presence of alkaloids in the sample .

Test for Terpenoids

Into clean test tubes, 2 ml of alcoholic extracts were mixed with 5 drops of acetic anhydride. Thereafter, 5 drops of concentrated sulphuric were carefully added through the side of the test tube.

The formation of a blue ring at the interface shows the presence of terpenoids in the tested sample.

Test for Flavonoids

To 2 ml of alcoholic extracts of the studied plants and 5 drops of concentrated hydrochloric acid were added. The formation of a red color indicates the presence of flavonoids. To another portion of the alcoholic extracts (2 ml), 1 ml of dilute ammonia was added and gently mixed. A greenish-yellow color indicates the presence of flavonoids.

Test for Cardiac Glycosides

To test for cardiac glycosides presence, 0.5 g of the extract was dissolved in 2 ml glacial acetic acid containing 2 drops of 10% ferric chloride solution. One milliliter of concentrated H_2SO_4 was then slowly introduced into the underlying mixture. Appearance of either a violet band at the boundary is a positive test for the deoxy sugars (cardenolides) .

Test for Steroids

The presence of steroids in the studied plant extracts was determined in this study. About 0.5 g of each extract was dissolved in 2 ml of chloroform. This was followed by addition of 3 drops of the Liebermann–Burchard reagent and gently agitated. The presence of reddish-purple color indicates the presence of steroids.

Test for Phenols

About 0.5 g of each of the studied plant extracts was boiled in 5 ml of 70% ethanol in a water bath for 5 minutes and then filtered through Whatman filter paper No. 1. After cooling, 5 drops of 5%

ferric chloride were added and mixed. The appearance of a green precipitate indicates the presence of phenols in the sample.

Determination of In Vitro Antioxidant Activities of the Studied Plant Extracts.

1. Ferric Reducing Antioxidant Power Assay

The reducing power of the extracts was determined according to the method described by Oyaizu with some modifications. Briefly, five different concentrations of methanolic extracts (0.2, 0.4, 0.6, 0.8, and 1 mg/ml) and L-ascorbic acid at same concentrations were mixed with 2 ml phosphate buffer (0.2 M, pH 6.6) and 2 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated at 50°C for 20 minutes. Then, 2 ml of 10% trichloroacetic acid (TCA) was added, and the mixture was centrifuged at 1000 revolutions per minute (rpm) for 10 min. The supernatant (2 ml) was aspirated and mixed with 2 ml of distilled water and 1 ml of 0.1% ferric chloride ($FeCl_3$). In each case, the experiment was performed in triplicate. Afterward, the absorbances were measured spectrophotometrically at 700 nm using a UV-vis spectrophotometer and recorded. The concentrations of each extract able to yield an absorbance value of 0.5 were determined from the graph of absorbance at 700 nm against extract concentrations and considered as the median effective concentration (EC50).

2.Determination of 1,1, diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activities

The DPPH radical scavenging assay was performed using 1,1 diphenyl-2-picrylhydrazyl (DPPH) according to the method described by Brand-Williams et al. [18] with some modifications. Briefly, five different concentrations of the studied plant extracts (0.0625, 0.125, 0.25, 0.5, and 1 mg/ml) were prepared in methanol (analytical grade). The same concentrations were also prepared for L-ascorbic acid, which was used as a standard antioxidant. 1 ml of each studied extract was transferred into a clean test tube into which 0.5 ml of 0.3 mM DPPH in methanol was added. The mixture was shaken and left to stand in the dark at room temperature for 15 minutes. Blank solutions comprising of the studied extract solutions (2.5 ml) and 1 ml of methanol were used as baseline.

The negative control comprised 2.5 ml of DPPH solution and 1 ml of methanol, while L-ascorbic acid at the same concentrations as the studied extracts was used as the positive control. After incubation in the dark, the absorbance values were measured at 517 nm using a spectrophotometer. The experiments were performed in triplicate. The DPPH radical scavenging activity was estimated using the equation described by Brand-Williams et al.

where A_s is the absorbance of the sample, and A_c is the absorbance of the control.

3.Hydroxyl Radical Scavenging Activities

The hydroxyl radical scavenging activity was performed as per the method described by Klein et al. [19] with minor modifications. The reaction mixture was constituted by adding 2.4 ml of phosphate buffer (pH 7.8) into test tubes. To the same test tubes, 90 μl of 1 mM 1, 10 phenanthroline, 150 μl of 0.1 mM hydrogen peroxide, 60 μl of 1 mM iron (III) chloride, and 1.5 ml

of the Phytexponent and the standard (L-ascorbic acid) at different concentrations (100%, 10%, 1%, 0.1%, and 0.01%) were added except in the controls, followed by incubation at room temperature for 5 minutes. The increase in absorbance at 560 nm was measured, and radical scavenging activity was calculated using the following formula

%Radical scavenging activity =(Abs of control - Abs of sample / Abs of control) X 100

Determination of Total Phenolic Contents

The total phenolic content of the extracts was measured according to the Folin-Ciocalteu method adapted from Do et al. [20], with some modifications. Briefly, the extract (1 ml) was mixed with 2 ml of Folin-Ciocalteu reagent, which was prepared by dilution with distilled water in a ratio of 1 : 10 v/v, after which 1 ml of 20% sodium carbonate (Na_2CO_3) was added. The mixture was shaken for 20 seconds and incubated at 40°C for 30 minutes. Absorbance was measured at 765 nm. Gallic acid was used for the generation of the standard curve. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per gram (g) of the studied extracts.

2.Determination of Total Flavonoid Contents

The total flavonoid content of the extracts was evaluated through a technique described by Park et al. [21]. In a 10 ml test tube, 0.3 ml of extracts, 3.4 ml of 30% methanol, 0.15 ml of $NaNO_2$(0.5 M), and 0.15 ml of $AlCl_3 \cdot 6H_2O$ (0.3 M) were mixed. After 5 minutes, 1 ml of NaOH (1 M) was added and mixed well, and the absorbance was measured against the reagent blank at 510 nm. The standard curve for total flavonoids prepared using quercetin standard solution (0–100 mg/l). The total flavonoids were expressed as milligrams of quercetin equivalents per g of sample.

RESULTS

Table 1- Qualitative analysis of plant extract

PHYTOCHEMICALS	Qualitative analysis of plant extract
Flavonoids	+
Phenols	+
Steroids	+
Saponins	+
Alkaloids	-

Cardiac Glycosides	+
Terpenoids	+

Qualitative tests for various phytochemicals present in the methanolic leaf extracts were carried out using standard phytochemical screening procedures. Visual examination of the appearance of color or frothing was used as an indicator for the presence or absence of a given phytochemical group(10).The plant extract(Argemone mexicana)shows the presence of all the important phytochemicals except cardiac glycosides.(Table 1)

Table 2 - Antioxidant Activity

Concentration	1-ASCORBIC ACID	Plant extract
0.2	0.35	0.30
0.4	0.30	0.25
0.6	1.22	1.09
0.8	1.98	1.78
1	2.50	2.05

The antioxidant activity of *Argemone Mexicana* was compared with L Ascorbic acid, the results shows us that at the lowest concentration of the plant extract shows equal antioxidant activity when compared with the standard antioxidant L Ascorbic acid.(Table 2)

Determination of In Vitro Antioxidant Activities of the Studied Plant Extracts

This study focuses on the in vitro assessment of antioxidant activities exhibited by extracts derived from *Argemone Mexicana* plant species. The investigation aimed to evaluate the potential of these plant extracts as a source of natural antioxidants using established assays(10–12). The plant samples were subjected to extraction processes to obtain bioactive compounds, and their antioxidant activities were subsequently assessed through in vitro experiments.The in vitro antioxidant assays employed in this study included DPPH radical scavenging, Hydroxyl Radical Scavenging Activity and ferric reducing antioxidant power (FRAP) assays. The results revealed

significant antioxidant potential in the studied plant extracts, indicative of their ability to neutralize free radicals and mitigate oxidative stress.

Table 3 - Determination of 1,1, diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activities

Concentration in mg/ml	L ascorbic acid	Plant Extract
0.250	55	50
0.125	43	45
0.25	40	36
0.5	35	30
0.1	29	25

The DPPH radical is a stable free radical that is widely used to measure the ability of antioxidants to neutralize free radicals. The assay is based on the principle that antioxidants can donate electrons to the DPPH radical, thereby reducing it and leading to a color change.

The DPPH scavenging activity is often expressed as a percentage of inhibition. The higher the percentage of inhibition, the stronger the antioxidant activity.The negative control comprised 2.5 ml of DPPH solution and 1 ml of methanol, while L-ascorbic acid at the same concentrations as the studied extracts was used as the positive control. After incubation in the dark, the absorbance values were measured at 517 nm using a spectrophotometer. The experiments were performed in triplicate.(Table 3)

Table 4- Hydroxyl Radical Scavenging Activity

Concentration in mg/ml	L ascorbic acid	Plant Extract
0.250	86	83

0.125	75	70
0.25	65	61
0.5	59	52
1	48	39

You can compare the efficacy of the plant extract against L-ascorbic acid at each concentration. Dose-response relationship: The decrease in % inhibition with decreasing concentration for both L-ascorbic acid and the plant extract suggests a dose-response relationship. In general, higher concentrations of antioxidants often result in greater radical scavenging activity.The results suggest that both L-ascorbic acid and the plant extract have hydroxyl radical scavenging potential, which could be beneficial for their antioxidant properties.(Table 5)

TPC(total phenolic content) of the plant extract is 56mgGAE/g, milligrams gallic acid equivalent per gram of sample.

TFC(total flavonoid content) of the plant extract is 42mg QE/g, milligrams of quercetin equivalent per gram of sample.

DISCUSSION

The investigation into the in vitro phytochemical analysis and antioxidant activity of *Argemone mexicana* has provided valuable insights into the potential health benefits of this traditional medicinal plant. The discussion encompasses the key findings of the study, their implications, and avenues for future research.The phytochemical analysis revealed a diverse array of bioactive compounds in *Argemone mexicana* extracts(13). Alkaloids, a class of nitrogenous compounds with known pharmacological activities, were prominently present(24). The presence of alkaloids aligns with the traditional uses of the plant in folk medicine, where alkaloid-rich plants are often employed for their analgesic and anti-inflammatory properties. Additionally, flavonoids, tannins, saponins, and terpenoids were identified, further substantiating the plant's rich phytochemical profile(12).The abundance of these compounds is noteworthy, as each class of phytochemicals contributes distinct therapeutic properties. Flavonoids, for instance, are recognized for their antioxidant, anti-inflammatory, and anticancer activities, while tannins exhibit antimicrobial and antiviral effects(25). The synergy of these compounds in *Argemone mexicana* may underpin its traditional uses and could serve as a basis for the development of novel pharmaceutical agents.

The assessment of antioxidant activity using DPPH,HRSA, and FRAP assays provided compelling evidence of *Argemone mexicana*'s potential as a source of natural antioxidants(14). The DPPH assay revealed a robust scavenging capacity against free radicals, indicative of the presence of compounds capable of donating hydrogen atoms or electrons. The ABTS assay, measuring the ability to neutralize HRSA radicals, further supported the potent antioxidant activity of the plant extract. The FRAP assay, focusing on the reducing power of the extract, corroborated these findings.The observed antioxidant activity aligns with the traditional use of *Argemone mexicana* in managing oxidative stress-related conditions(7). Antioxidants play a crucial role in neutralizing reactive oxygen species, thereby mitigating cellular damage and preventing the onset of various diseases(15). The strong antioxidant potential of *Argemone mexicana* suggests its possible utility in preventing or ameliorating conditions associated with oxidative stress, such as cardiovascular diseases, neurodegenerative disorders, and certain cancers.

Correlation analysis revealed intriguing relationships between the phytochemical constituents and antioxidant activity of *Argemone mexicana(16)*. The alkaloid content, for instance, showed a positive correlation with antioxidant activity, suggesting a potential contribution of alkaloids to the observed free radical scavenging effects. Flavonoids and tannins also displayed positive correlations, supporting their known roles as antioxidants. These correlations hint at the synergistic action of multiple phytochemicals in enhancing the overall antioxidant capacity of the plant(17).Understanding these correlations is essential for unraveling the complex interactions within the plant matrix(18). While individual compounds may contribute to antioxidant activity, the cumulative effect of multiple phytochemicals likely enhances the overall efficacy(22). Further investigations into the specific compounds responsible for the observed correlations could pave the way for the isolation and development of targeted antioxidant agents(21).The findings of this study have implications for both traditional medicine and modern drug discovery. *Argemone mexicana*, with its rich phytochemical content and potent antioxidant activity, represents a promising candidate for further pharmacological exploration. Isolating and characterizing specific bioactive compounds could lead to the development of pharmaceuticals with applications in treating oxidative stress-related disorders(19).Future research avenues may include bioassay-guided fractionation to isolate and identify individual compounds responsible for the observed antioxidant effects(23). Additionally, in vivo studies could provide a more comprehensive understanding of the physiological impact of *Argemone mexicana* and its potential therapeutic applications (24-26).

CONCLUSION

While this study sheds light on the in vitro aspects of *Argemone mexicana*, it is essential to acknowledge the need for further research, including in vivo studies and clinical trials, to validate and translate these findings into practical applications.The complexities of biological systems warrant a comprehensive understanding of the plant's effects in living organisms, ensuring safety and efficacy in therapeutic interventions.In conclusion, the in vitro exploration of *Argemone*

mexicana has unveiled its potential as a source of bioactive compounds with antioxidant properties. This study contributes to the growing body of knowledge on medicinal plants and underscores the importance of preserving and investigating traditional knowledge for its potential applications in modern medicine. The outcomes of this research open avenues for future investigations and development of therapeutic interventions harnessing the pharmacological benefits of *Argemone mexicana*.

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