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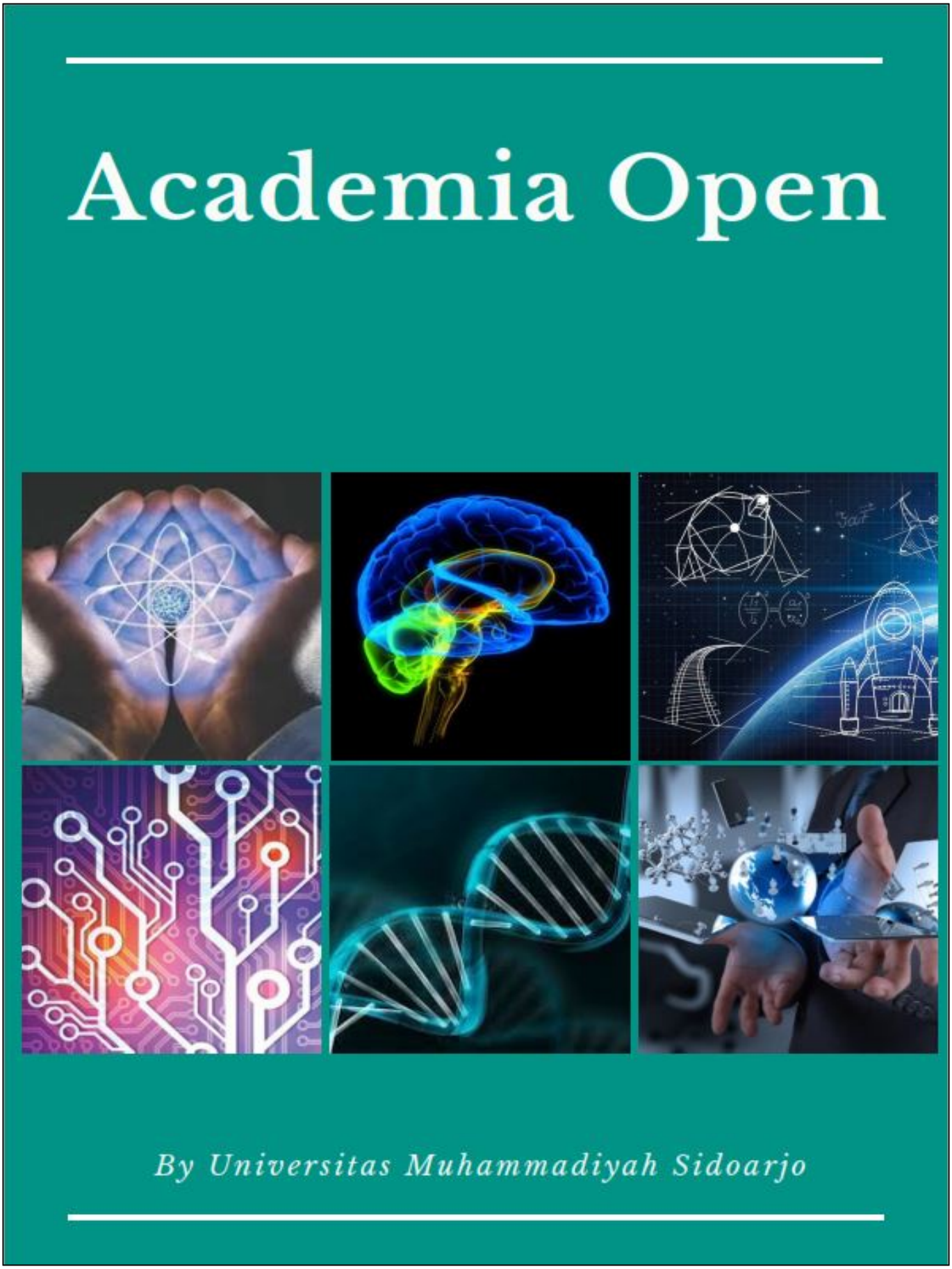

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Phytochemicals and Antioxidant Potential of Amaranthus spinosus Seed Extracts

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Abstract

General Background: Amaranthus spinosus, a member of the Amaranthaceae family, has long been valued in culinary and medicinal traditions due to its diverse bioactive compounds. **Specific Background:** Previous studies have highlighted its phenolic content and antioxidant potential, yet limited research has compared solvent efficiency in extracting these compounds. **Knowledge Gap:** The influence of solvent polarity on phytochemical diversity and antioxidant activity of A. spinosus seeds remains underexplored. **Aims:** This study aimed to investigate the phytochemical composition and antioxidant capacity of aqueous and alcoholic extracts of A. spinosus seeds. **Results:** Both extracts contained polyphenols, flavonoids, terpenoids, alkaloids, and saponins, though glycosides were exclusive to the aqueous extract, while carotenoids and cardiac glycosides were unique to the alcoholic extract. Antioxidant assays revealed stronger radical scavenging activity in the alcoholic extract (88%) than in the aqueous extract (75%), though both were below the ascorbic acid standard. **Novelty:** By demonstrating solvent-specific extraction patterns, this study underscores alcohol as a superior medium for isolating antioxidant constituents in A. spinosus seeds. **Implications:** These findings validate the traditional use of A. spinosus and suggest its potential application as a natural antioxidant in pharmaceutical and food industries

Highlights:

- Alcoholic extract showed higher antioxidant capacity (88%) than aqueous (75%).
- Solvent choice influenced phytochemical diversity and yield.
- Supports traditional medicinal use and potential food/pharma applications.

Keywords: Amaranthus spinosus, Phytochemicals, Antioxidant Activity, Solvent Extraction, Bioactive Compounds

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Introduction

A. spinosus is a plant commonly utilized for both culinary and traditional medicinal purposes for an extended period. It is a member of the Amaranthaceae family[1]. widely cultivated in India, Sri Lanka, and other tropical regions is widely used in Ayurveda and is reported to have diverse medicinal properties, but is present on most continents as an introduced species and sometimes a noxious weed, It can be a serious weed of rice cultivation in Asia. The fruit contains several seeds [2] [3]. The majority of seeds exhibit a robust structure and present predominantly in tan or off-white coloration, uniformly encased by a protective outer layer [4]. A. spinosus is extensively cultivated in India and various warm climates globally for its culinary and medicinal applications. Both the fruits and aerial parts of the plant are frequently utilized as vegetables. The fruits are reported to possess agreeable, refrigerant, emollient, diuretic, sedative, and tonic properties, demonstrating therapeutic benefits for conditions such as burns, scalds, inflammations, abscesses, boils, migraine, and neuralgia [5]. Globally, A. spinosus is incorporated into human nutrition in diverse culinary preparations, including soups, purees, jams, and pies [6].

In a separate study, the constituents of Acanthopanax spinosus's seed oil showed a significant capacity to mitigate oxidative stress, six of the most significant compounds were identified as being phenolic in nature [7]. These included syringic acid, along with tocopherols and sterols, particularly δ-tocopherol and β-sitosterol, respectively, [8]. Previous studies have demonstrated that the seeds of A. spinosus have a high concentration of total Phenolic compounds and have a significant capacity to antioxidant, as determined by various chemical tests [9]. Additionally, the shells and seeds have been incorporated into baked goods that enhance their antioxidant properties and overall chemical composition. Given the chemical composition of these byproducts and their significant antioxidant and antibacterial properties, they have the potential to be utilized effectively in the creation of natural food preservatives [10]. Traditionally, this plant has been utilized in multiple countries, including India, China, Brazil, Yugoslavia, and the U.S., for its anti-inflammatory, immunomodulatory, and antibacterial properties. Additionally, it's been employed for its anti-lipidemic and anti-insulitic properties [11] [12]. The frequency of A. spinosus in diverse traditional medicinal systems. Several diseases had the investigator focus on this plant. This research aims to explore the first physicalchemical and chemical properties of the seeds of Cucurbita maxima, this information is important in the pharmacological properties of the seeds.

Methods

A. Extract Preparation

The seeds of *A. spinosus* were sourced from a local market in Helal city. Following collection, the seeds were separated from residual plant material and allowed to air-dry at ambient temperature. Once dried, the seeds were mechanically ground to achieve a homogenous powder[13]. Twenty-gram samples of the powdered seeds were accurately weighed, and 200ml of methanol was added to each. The resulting mixtures were subjected to maceration for 24 hours under continuous agitation using a shaker at room temperature. Subsequently, the materials underwent filtration using Whatman grade 1 filter paper. The methanol solvent was then removed by evaporation at room temperature under a fume hood. The obtained extracts were retained for subsequent analyses [14].

1. Prepare Extract using Soxhlet Apparatus

The seeds were pulverized, subjected to a 40-mesh filter, and extracted using a Soxhlet apparatus in succession with petroleum ether (60-80 °C), ethyl acetate, and alcohol. Prior to each extraction with the subsequent solvent, the powdered material was desiccated in a hot air oven at temperatures below 50°C. Each extract was further concentrated by evaporating the solvent to dryness using a water bath. All extracts were preserved at 4 °C for qualitative analysis and anti-oxidants investigations[15] [16].

B. Chemical Content

Confirmatory qualitative phytochemical screening of the plant extracts was conducted to identify the major classes of secondary metabolites present. These analyses targeted tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids. The screening procedures were performed following established, conventional protocols**.** [17] [18].

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C. Antioxidant Assay

The antioxidant activity of the *A. spinosus* seeds extract was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, with minor modifications based on the method described by Villano et al. [19]. Serial dilutions of the A. spinosus extract (20, 40, 60, 80, and 100 mg/µg in ethanol), with a volume of 1 ml each, were mixed with an equal volume of DPPH solution. The absorbance of the resulting mixtures was measured at 517 nm after a 1-hour incubation period in the dark at room temperature. The DPPH radical scavenging capacity of the sample was calculated using Equation :

$$\text{DPPH scavenge effect} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100 \quad (1)$$

D. Statistical Analysis

Results are presented as Mean ± SD of twelve distinct samples, each duplicated. The Statistical Analysis Systems (SAS, 2019) software was employed for data analysis [20].

Results and Discussion

Table 1 compares the qualitative composition of extracts derived from the seeds of A. spinosus in terms of their extraction solvents. Water and alcohol are both used as solvents in the extraction of these compounds. The amount (+) or lack of different classes of metabolites with a secondary origin was assessed in each extract. Both water and alcohol-based extracts have successfully produced extracts that contain polyphenols, flavonoids, terpenoids, alkaloids, and saponins. This suggests that these compounds have a degree of solubility in both polar (water) and intermediately polar (alcohol) solvents. The presence of these bioactive compounds is in line with previous studies that have documented the antioxidant and other biological properties associated with *A. spinosus*.

However, significant discrepancies were noted. Glycosides were observed in the water-based extract, but not in the alcohol-based extract. This implies that the glycosidic compounds in the seeds have a preference for water and are not effectively extracted by alcohol. On the other hand, cardiac glycosides and carotenoids were only observed in the alcoholic extract, this suggests that they have a high preference for the less polar solvents found in alcohol. Anthraquinones were present in both extracts. These results demonstrate the significance of solvent choice in the extraction of chemical compounds, different solvents have different capabilities to dissolve particular classes of substances. For a comprehensive chemical analysis of the extracts from A. spinosus seeds, a variety of solvents with different polarities must be used. This is because it is necessary to have a broad spectrum of bioactive components in order to successfully extract these components from the seeds. Further studies that quantify the concentrations of these specific compounds in each extract are necessary to associate the observed biological activities with them.

Table 1. The composition of chemical substances in extracts that are alcoholic prepared by the three methods from *A. sponusis* seeds.

Phytochemical	Water extraction	Alcohol extraction
Polyphenols	+	+
Alkaloids	-	-
Flavonoids	+	+
Terpenoids	+	+
Glycosides	+	-
Alkaloids	+	+
Anthraquinone	+	+
Cardiac glycoside	-	+

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Saponin	+	+
Carotenoids	-	+

+ = Present - = Absent

Studies have identified several chemical compounds with a plant origin. These chemical compounds are classified into three large categories: alkaloids, flavonoids, Phenols, polysaccharides, tannins, saponins, terpenoids, and proteins. This implies that A. spinosusis is not only utilized as a food source in multiple communities around the world, but it also has the potential to serve as a crucial raw material for the development of pharmaceuticals in the pharmaceutical industry. Our results are in line with those of Kabashi et al.. [21]. According to their research, the ethanolic extract of A. spinosus seeds exhibited significant antioxidant activity. The study results showed that a number of different bacteria, such as P. aeruginosa, A. niger, C. albicans, E. coli, and B. subtilis, possess high levels of antibacterial activity. Due to its high content of plant components, *A. spinosusus* has the potential to be used in the treatment of a variety of ailments affecting the body. To be honest, Kayley and a few others stated in their review that *A. spinosusus* has anti-inflammatory activity, while Abu Elila et al. [22] stated that the seeds exhibited anticancer function. According to traditional medicine, the seeds of this plant are used to treat irritable bladder syndrome and prostate enlargement in the early stages of both conditions. In the presence of high-hydroxyl phenolic compounds, also known as flavonoids, these effects are associated with the formation of complexes with the bacterial cell wall, disrupting the integrity of the cell wall and ultimately leading to the death of the microbe [23]. The presence of saponins in A. spinosus not only contributes to its medicinal effectiveness but also makes it suitable for use in industrial applications, such as the manufacture of detergents and active ingredients in services.

1. Antioxidant Activity

Figure 1 illustrates the percentage of the DPPH radical that is removed (%) using different extracts and a standard. The x-axis represents the type of sample: "EX Water" (aqueous extract), "EX Alcohol" (alcoholic extract), and "Ascorbic (mg/mL)" (ascorbic acid solution, which is used as a positive control). The y-axis represents the percentage of radical destruction, which is0% to 100%. The aqueous extract ("EX Water") has a radical removal of approximately 75%. The alcoholic extract ("EX Alcohol") has a much greater removal rate, reaching around 88%. The ascorbic acid concentration standard ("Ascorbic (mg/mL)") has the greatest capacity for removal of ascorbic acid among the tested samples, it is almost 90% effective. Error bars are positioned on each bar, these represent the standard deviation or standard error of the measurements. The relatively small size of the error bars in this figure indicates that the samples are reproducible within each group. Overall, the data indicates that both aqueous and alcoholic extracts of Acanthopanax spinosus seeds have antioxidant properties, this is demonstrated by their capacity to inhibit the release of DPPH radicals. The alcoholic extract has a significantly greater scavenging ability than the aqueous extract, although both are still below the standard concentration of ascorbic acid in the conditions tested. This implies that the compounds responsible for the antioxidant properties are more effectively isolated using alcohol as a solvent..

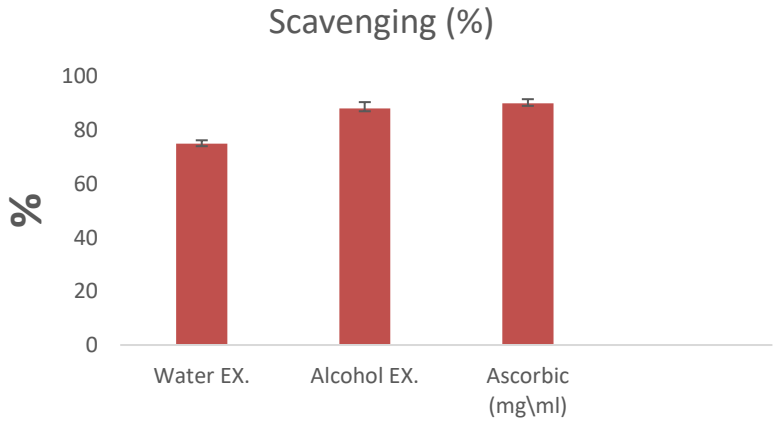

Figure 1. The antioxidant activity of LSD0.0=3.547

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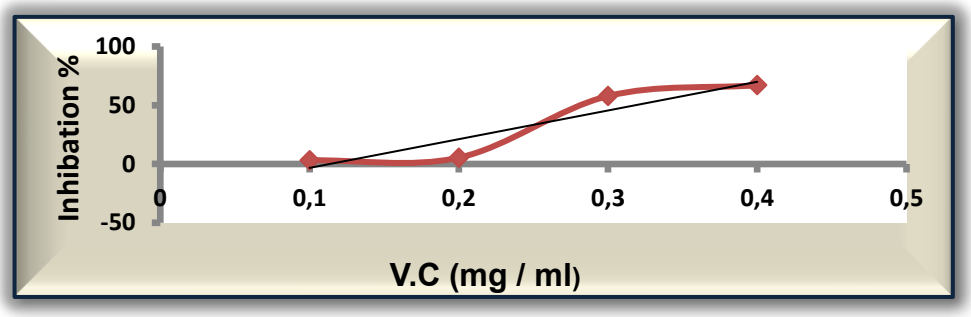

Figure 2. Show the curve of Ascorbic (V.C)

These findings are in agreement with other authors who have documented the antioxidant properties of various species of A. spinosus, particularly the seeds, using multiple methodological approaches. Researchers [24] studied the capacity for antioxidants in the seeds. They discovered that the methanolic extract they employed had a significant effect on reducing the amount of TBARS in the testicular tissue of albino mice. consonant with the findings of a different investigation [25], pumpkin seeds and peels extracted using a variety of solvents demonstrated significant potential for scavenging DPPH radicals. Furthermore, peel samples exhibited the highest inhibition rate, reaching 71.0 ± 0.97% DPPH radical inhibition. The most effective solvent was 70% ethanol, which was also the most efficient solvent. Furthermore, compared to raw burgers, improved fat stability during storage and antioxidant properties were found in chicken patties containing pumpkin seeds in a study evaluating the combination of seeds with food products [26]. Given the fact that the plant parts used and the procedures employed were different, the results found in the literature cannot be compared to the results presented in this article. During the course of this research, only cell-based techniques were used to more accurately simulate the mechanisms involved in biological systems. According to the information currently available, phytochemical studies have been conducted on A. spinosus using a variety of solvents, and the therapeutic activity of this plant has also been investigated. Ethnobotanical research has doubled in recent years; however, the loss of biodiversity due to climate change, encroachment on forests and other habitats, and other factors may be contributing to a slowdown in the search for plant-based medicines [27]. Furthermore, research has demonstrated that plant-derived medicines are not only cost-effective but also safe and environmentally friendly. Consequently, there is an urgent need to conserve and study A. spinosus for its potential medicinal value [28] [29]. According to the results of the current study, this plant could be used in the pharmaceutical industry for drug development.

Conclusion

A. spinosus seed extracts (aqueous and alcoholic) demonstrated the presence of active compounds (polyphenols, flavonoids, etc.) and antioxidant activity in the DPPH assay. The alcoholic extract significantly outperformed the aqueous extract in this activity, but both were lower than the standard ascorbic acid. The choice of solvent indicates its influence on the extraction of specific compounds contributing to the biological activity. The results confirm the antioxidant potential of this plant's seeds and support its traditional use.

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