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REVIEW ARTICLE

Impact of Solvents on *Cucurbita Maxima* Leaf Antioxidant Activity: An Examination of Extraction Techniques and Phytochemicals

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ABSTRACT
Bioactive constituents with high potential antioxidant activity are found in Cucurbita maxima leaves. Phytochemical yield is influenced by the solvent efficiency of the solvent used which can enhance flavonoid and polyphenol recovery when it is a polar solvent (ethanol, methanol) but lipid soluble antioxidant solvent recovery when it is a non-polar solvent (hexane, chloroform). The studies show that ethanol is not toxic and thus presents a vitally important ingredient in nutraceutical applications. The challenges include the toxicity of solvent, lack of standardization, and the necessity of green extraction techniques. This can bring up future research on extracting bioactive compounds by optimizing extraction parameters and exploring eco-friendly solvents to improve the recovery of these bioactive compounds.
Keywords: *Cucurbita maxima, antioxidants, solvent extraction, bioactive compounds, phytochemical yield, green extraction, nutraceuticals.*

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INTRODUCTION

Pumpkin, known scientifically as *Cucurbita maxima* is a common vegetable of the Cucurbitaceae family which is regarded as a nutritional and medicinal precious metal. *Cucurbita maxima* leaves are rich in an array of phytochemicals including flavonoids, phenolics, tannins, alkaloids, and carotenoids, and possess some pharmacological properties[1]. Antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective activity of these bioactive compounds. *Cucurbita maxima* leaves have been traditionally used to control Oxidative stress-related disorders in ethnopharmacology and can be considered a good botanical resource [2].

Reactive oxygen species (ROS) and free radicals are neutralized by antioxidants and prevent oxidative damage which is related to chronic diseases, for example, cardiovascular disorders and cancer [3]. Phytochemicals in *Cucurbita maxima* leaves serve as protectants against oxidative stress-related cell damage, support pharmacognostic application, and reduce lipid peroxidation as well as enhance enzymatic activity.

Plant matrices are a critical manipulation point for obtaining the isolation of bioactive secondary metabolites. Bioavailability, solubility, and stability of phytoconstituents depend on the selection of solvent used [4]. The efficiency of extracting phenolic acids flavonoids and terpenoids is a function of solvent polarity, and therefore they will affect the antioxidant capacity of the extract.

Aim

In summary, this review aims to appraise the influence of various solvents regarding the antioxidant activity of *Cucurbita maxima* leaves with special regard to their phytochemical composition and pharmacological importance.

Objectives

- To show the extent to which solvent polarity affects the extraction yield of the bioactive compounds.
- To compare antioxidant activity based on different solvent extraction methods.
- To identify optimal solvent systems for maximizing the therapeutic potential of *Cucurbita maxima* leaves.

PHYTOCHEMICAL COMPOSITION OF *CUCURBITA MAXIMA* LEAVES

Overview of Major Phytochemicals

Total flavonoids, polyphenols, tannins, and alkaloids were found in *Cucurbita maxima* leaves and therefore for the pharmacological activity of the leaves. However, flavonoids belong to one of the largest groups of bioactive compounds with free radical scavenging and cell-protecting characteristics [5]. The phenolic acids and their derivatives can act as an effective antioxidant and donate hydrogen atoms to other molecules to combat free radicals [6]. In addition, they participate in much radical quenching and metal chelation that is efficient in the prevention and control of oxidative stress [7]. Furthermore, there are other secondary metabolites found in *Cucurbita maxima* leaves, and from the point of view of pharmacognosists, they have cytoprotective and anti-inflammatory properties [8].

Role of Phytochemicals in Antioxidant Activity

The phytochemicals contribute mainly to the antioxidant ability of the *Cucurbita maxima* leaves. Flavonoids and polyphenols have been studied to arrest lipid peroxidation and repair radical-induced cellular damage [9]. Antioxidant effects of tannins can be through the activity of enzymes such as superoxide dismutase and catalase, which are involved in oxidation stress [10]. Alkaloids are strong antioxidants that halt the free radical chain reactions and inflammatory processes by mediating cellular signaling pathways [11]. Due to the combined effect of these bioactive compounds, there is potential to use *Cucurbita maxima* leaves as a natural antioxidant source for the nutraceutical industry [12].

Factors Influencing Phytochemical Composition

The phytochemical content of *Cucurbita maxima* leaves reflects the influence of various intrinsic and extrinsic factors. The synthesis of secondary metabolites is also influenced by the following factors; the type of soil, temperature, and water content of the plant [13]. Phytochemical content is also influenced by the plant maturity wherein young leaves show higher flavonoid and polyphenol content as compared to mature leaves [14]. Also, other handling practices during storage, processing, and packaging affect the stability and bioavailability of these bioactive compounds [15]. This knowledge is important for the optimization of both extraction methods and pharmacology of *Cucurbita maxima* leaves.

EXTRACTION METHODS AND THEIR IMPACT ON ANTIOXIDANT ACTIVITY

Overview of Solvent Extraction

Solvents are crucial in the extraction of bioactive constituents from *Cucurbita maxima* leaves and as the polarity of solvent augments the augments the extraction efficiency [16]. It has been reported that ethanol and methanol improved the extraction of flavonoids, tannins, and phenolic acid with high antioxidant activity [17]. The intermediate polarity extracts hydrophilic and lipophilic compounds with acetone and ethyl acetate, increasing the yield of phytochemicals [18]. For carotenoids and terpenoids, extracting carotenoids is effective for terpenoids, and the latter contributes to free radical scavenging [19]. It is important to select an appropriate solvent system for extracting compounds in pharmacognosy to promote bioavailability and stability.

Comparative Analysis of Different Solvent Systems

Since ethanol and methanol can disrupt the hydrogen bonds that maintain the integrity of polyphenols and flavonoids in plants, ethanol and methanol are preferred in pharmacognostic research [20]. Despite being used widely, the efficiency of water as a solvent for highly complex polyphenolic structures is low [21]. This is the most preferred technology because it is non-toxic, as such, it can be used in nutraceutical applications[1]. Lipid-soluble phytochemicals are efficiently extracted from plants using hexane and chloroform; however, toxicity restricts their use in pharmaceutical applications [22]. The polar and non-polar antioxidants are extracted by ethyl acetate according to a balance [23].

Antioxidant activity is enhanced by optimizing the extraction protocols. Polyphenol recovery is improved by ethanol mixtures with ethanol, and flavonoid yield is increased using aqueous ethanol [24]. This temperature control prevents enzymatic degradation and ultrasonication improves the solvent penetration that facilitates bioactive compound extraction [25]. The solvent extraction technique needs standardization in pharmacognosy for consistency in the formulation of antioxidants.

ANTIOXIDANT ACTIVITY EVALUATION METHODS

DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Assay

The method used for the pharmacognostic studies of the plant extracts for the free radical scavenging activity is the DPPH assay, so such assay becomes an indispensable method of study. The basis of this method is the fact that antioxidant compounds can donate electrons or hydrogen atoms to neutralize the DPPH radical, leading to color changes from deep violet to yellow [26]. Spectrophotometric at 517 nm, the degree of discoloration is measured and it is quantified the antioxidant capacity [27].

Solvent polarity, extract concentration, and incubation time affect the efficiency of the DPPH assay. Only modifications in incubation time have been proposed to increase the accuracy, as incubation with reactive oxygen species (ROS) for a prolonged time can lead to discrepancies in measurements of antioxidant potential. In that case, the reaction mechanism is either a hydrogen atom transfer (HAT) or an electron transfer (ET) process, depending on the concentration of phenolic compounds in the extract [28].

Thus, assay conditions such as pH, temperature, and solvent composition are optimized for reproducibility and accuracy to measure the antioxidant potential of bioactive constituents

FRAP (Ferric Reducing Antioxidant Power) Assay

FRAP is the widely used technique in pharmacognostic research for antioxidant potential evaluation of biologically active compounds due to their capacity to reduce ferric ions (Fe^{3+}) to ferrous forms (Fe^{2+}). It happens in an acidic medium and generates a product ferrous-tripyridyltriazine (Fe^{2+}-TPTZ) complex with a 593 nm colorimetric detectable. After the color change, the intensity of the color change is directly proportional to the reducing power of the sample and can be used to quantify antioxidant capacity.

Since polyphenols, flavonoids, and ascorbic acid are hydrophilic antioxidants, the main advantage of this method is its capability to estimate hydrophilic antioxidants as the FRAP assay is primarily effective for the case of hydrophilic antioxidants [30]. Nevertheless, its limitation is that it does not detect lipophilic antioxidants where alternative assays are required to give accurate evaluation [5,31]. To date, the method has been exploited in the analysis of the antioxidant activity of plant extract, fruit-based nutritional supplements, and packaged juices with bioactive phytochemicals. Reaction time and temperature are optimized to achieve higher reproducibility and precise quantification of redox-active compounds.

ABTS (2,2′-Azino-Bis(3-Ethylbenzothiazoline-6-Sulfonic Acid)) Assay

The ABTS assay has become a commonly used method of assay in pharmacognosy to quantify the antioxidant capacity of plant extract and other bioactive substances. $ABTS^{+}$ radical cation is generated in this method with a characteristic blue-green color. Electron donation or hydrogen atom transfer to $ABTS^{+}$ decreases the absorption at 734 nm, converting the reduction product and reacting with an antioxidant [32]. The colorimetric change is subsequently used to quantitatively determine the radical scavenging activity of samples of diverse sorts.

The ABTS assay is a useful assay to measure hydrophilic and lipophilic antioxidants because they are more versatile than other assays used such as DPPH [33]. This has been used widely to investigate the antioxidant potential of fruits, vegetables, and spices, or to explore the phytochemical composition of such commodities; the information gained from such study is pretty useful [34]. Second, it has been demonstrated that the ABTS assay also extracts the total antioxidant capacity of Althaea and Hibiscus species and other plants [35]. Assay conditions are optimized to maximally increase the sensitivity in the context of bioactive phytochemicals so that the bioactive phytochemicals can be accurately evaluated [36].

ORAC (Oxygen Radical Absorbance Capacity) Assay

The ORAC assay method has wide applications of pharmacognosy for antioxidant potential evaluation of various bioactive compounds. The chemical reaction captured by this assay is the ability of an antioxidant to scavenge peroxyl radicals formed through the thermal decomposition of azo compounds. The probe (fluorescein) uses its fluorescence to map the probe concentration, and its fluorescence decays in the assay due to oxidation of the probe with reactive oxygen species (ROS). The radical scavenging capability of the sample under test can be inversely related to the amount of fluorescence loss [37].

The major advantage of the ORAC assay is that it can compare the kinetic parameters of fast-acting and long-acting antioxidants [38]. The accuracy of assessment for phenolic compounds and of the reactions with reactive oxygen species is improved [39]. Finally, the most recent improvements in the assay protocol have been shown to provide higher accuracy of the assay which is important, particularly in the estimation of hydroxycinnamic acids in fruit juices [40]. Additionally, ORAC has also been used in nanomedicine as a source of knowledge of the antioxidant mechanism of plant-derived extracts [41].

COMPARATIVE REVIEW OF STUDIES ON SOLVENT EFFECTS

Summary of Past Research Findings

Studies in the past have been conducted from 2020 to 2025 about the extraction efficiency of a couple of bioactive compounds extracted from different plant materials and selections of the solvents involved in the extraction [20]. The research has shown that the polarity of the solvents can affect a yield and a phytoconstituent composition. Studies related to thermosensation-assisted extraction of fennel revealed that phenolic compounds were extracted better with ethanol-water mixtures as compared to conventional methodologies. Research like that on Xanthium spinosum L. revealed that the phytochemical profile of the extract of this plant is influenced by its choice of solvent and, thus, by the biological activity as well [42].

Trends in Solvent Efficiency for Antioxidant Extraction

Some rootstocks such as ethanol and methanol extract polyphenols, flavonoids, and alkaloids effectively, and aqueous mixtures increase the solubility of hydrophilic antioxidant [43]. Lipophilic compounds, including terpenoids and carotenoids, may be dissolved in appropriate nonpolar (e.g. hexane) solvents. Current research indicates that deep eutectic solvents make the extracts more potent antioxidants while decreasing their toxicity [37].

Variations Based on Extraction Techniques

Techniques such as maceration, Soxhlet extraction, and ultrasound-assisted extraction (UAE) affect the extraction efficiency. The highest antioxidant activity was found with UAE combined with Soxhlet extraction because it exhibited improved mass transfer [44]. Enhanced recovery of phenolics in fennel has been achieved using harmonization, which outperformed conventional maceration [45].

CHALLENGES AND FUTURE RESEARCH DIRECTIONS

Limitations of Current Studies

However, little standardization exists in the extraction parameters such as the solvent composition, temperature, and extraction time during bioactive compound extraction research today. Variations in methodology lead to inconsistencies in the phytochemical profile and biological activity of extracts. Furthermore, conventional solvent-based extractions make use of toxic and environmentally hazardous solvents that preclude their application in pharmacognosy, functional foods.

Need for Green Extraction Methods

An increase in sustainability and a decrease in toxic solvent residues is possible by transitioning to green extraction technologies. Supercritical fluid extraction and usage of deep eutectic solvents (DES) have been investigated because of their ability to extract the secondary metabolites with minimum environmental impact.

Future Scope in Functional Food and Pharmaceutical Applications

Subsequent future studies' focus should be directed toward enhancing green extractions for use in functional food products and pharmaceuticals. It may also be applied to increasing the solubility of polyphenols, flavonoids, and alkaloids opening the way for the development of more efficient nutraceuticals.

CONCLUSION

This has shown that, probably the choice of the solvent and other conditions that surround extraction play's key role in the extraction of the bioactive compound. The instituted studies have shown that some efficient methods such as supercritical fluid extraction and deep eutectic solvents enhance the yield and purity of phytochemicals while minimizing or having minimal impacts on the environment. As a result, the identification of suitable solvents for the extraction of secondary metabolites is a vital factor in the extraction efficiency. Green solvents also contribute to creating a green environment by reducing toxicity in processes and methods used in pharmacognosy. Further research should also aim further improve of large-scale synthesis of green solvents for functional foods and pharmaceutical applications, besides the antioxidant and therapeutic efficiency of these solvents as well as other horizons of sustainability.

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