Research Article

In Vitro and *In Silico* Study of Antioxidant Effect on *Raphanus sativus* Microgreens and Mature Leaf

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ABSTRACT

The family Brassicaceae includes *Raphanus sativus* (radish). It has favorable pharmacological and nutritional properties. *Raphanus sativus* microgreen has been produced for the current investigation. Microgreens (dry and wet) and mature leaf (dry and wet) extracts' phytochemical components, biological characteristics, total phenolic content, and radical scavenging activity were compared. *R. sativus* methanolic extracts were examined for their phytochemical content and antioxidant capabilities (using the FC and DPPH assay methods). *R. sativus* microgreen dry methanolic extract shown more potential activity than other samples. The results of a molecular docking study conducted with CB-Dock (Cavity-detection guided blind docking) indicates that the phytochemicals quercetin, epicatechin, 3-hydroxy-beta-ionone, and P-coumaric acid has more efficiency than gallic acid, the positive control.

Keywords: *In-vitro* Antioxidant assay, DPPH, *Raphanus sativus*, Microgreen, Molecular docking, CB dock.

INTRODUCTION

An antioxidant is a substance that prevents other substances from oxidizing. By neutralizing the harmful effects of free radicals, which are organic byproducts of cell metabolism, they defend the vital cell components [1]. Free radicals are toxic byproducts created by cells as they digest food and respond to their surroundings. Oxidative stress can happen when the body is unable to adequately eliminate and process free radicals. Cells and physiological functions may be harmed by this. Reactive oxygen species (ROS) is the name given to free radicals. The "oxidative stress" that free radicals, which can destroy cells, can cause is helped to prevent by antioxidants [2].

Free radicals are species that are capable of independent existence and contain one or more unpaired 37 or 30 electrons. They interact with other molecules by accepting or donating electrons, and they are also involved in many pathological conditions where they have a positive impact on human health. Specifically, they can help people withstand or resist diseases like heart problems, lung damage, inflammation, and other conditions. Since free radicals are highly unstable, when their levels rise in the body, they can harm cells and tissues and possibly contribute to a number of disorders [2].

According to Tan, L et al., 2020, oxidative stress produces substantial cell damage that results in a various disease, including Parkinson's diseases, Alzheimer's diseases, cancer, arthritis, and neurological disorder [3]. Oxidative stress is a recent idea that has gained significant traction in the medical community during the last three decades.

Raphanus sativus, the scientific name of the radish, and the three species that make up the genus Raphanus are both members of the Brassicaceae family [4]. It is made up of leaves, which are widely consumed vegetables that are included in the human diet, but it is uncommon among some populations [5]. Radish is employed as a common domestic remedy for a variety of ailments, including roots, flavoring vegetables, etc., in Unani, Greeko-Arabic, and Indian folk medicine. According to Shukla, S., et al., it acts as a good remedies for indigestion, gastric pain, rectal prolapsed, gallstones, liver disorders, and jaundice [6].

Researchers and the pharmaceutical industry are interested in the potential use of radish as a source of bioactive compounds with clinical and health implications in diseases like hypertension, cardio metabolic disorders, and as an anti-diabetic, antioxidant, and anti-microbial agent [7].

Humans' diets that are rich in plants have several positive health effects. It lowers the risk of several diseases, including cancer, cardiovascular diseases, neurological disorders, and age-related issues [8].

Vegetable greens known as microgreens are picked right before one set of real cotyledon leaves has formed. They are quickly gathered after sprouting and utilized as a nutritional supplement, visual enhancement, and flavor and texture enhancement.

Compared to their mature counterparts, microgreens have higher concentrations of nutrients and health-enhancing micronutrients. In order to identify the phytochemical components, the current study is designed with the goal of producing microgreens. To further explore the inhibitory mechanism, a study on the antioxidant effects of radish microgreen, mature leaves, and in-silico was conducted.

MATERIALS AND METHODS

Micro green production

R. sativus seeds were purchased from Shilpa Hi- Tech Seeds in Hebbal, Bangalore, Karnataka, India and it's used for the micro green production.

Sample collection

Microgreen leaves, which are produced in trays, and mature leaves, which are purchased at local market. The samples were gathered, thoroughly cleaned, and water washed (wet sample). In order to get a dry sample, the leaves were left to dry in the shade of the sun and powder with mortar and pestle.

Extraction

After making a fine paste (1:10) by combining leaf powder (microgreen 20g and mature 20g) with methanol, an extraction procedure known as sonication is used. The extract was collected and centrifuged at 5,000 rpm for 10 minutes. Pure extracted samples were collected and used in further analysis [9].

IN-VITRO ANTIOXIDANT ASSAY

Determination of total phenolic content by Folin-Ciocalteu (FC) method:

The Folin-Ciocalteu method, as described by B. Aruna et al., 2017 with some modifications, was used to determine the total phenolic content [10]. Different concentrations of the plant extract (20 µl to 100 µl) were added to various amounts of distilled water after being diluted in a phosphate buffer. For a brief while, the tubes were spined. To all of the test tubes, 150 µl of FC reagent was added. 500 µl of 20% sodium carbonate was added to each tube after a 5–10 minute incubation period at room temperature. For an hour, tubes were kept in the dark for incubation. A colorimeter and UV spectrometer were used to detect the absorbance at 517 nm. The amount of total phenolic content in each sample was measured in mg of gallic acid equivalents (GAE).

Antioxidant activity- 2,2 Diphenylpicrylhydrazyl (DPPH) method

The DPPH method, as reported by Tuba Ak and İlhami Gulcin was used to conduct the in-vitro antioxidant assay with a few modifications [11]. Different amounts of 0.1 M tris Hcl were added along with various concentrations of the plant extract (20 µl to 100 µl) diluted in a phosphate buffer. After that, 1ml of the DPPH solution is added to each test tube 1-5. In order to do the positive control (ascorbic acid), 800 µl of 0.1 M tris Hcl, 1 ml of DPPH solution, and 200 µl of plant-free ethanol were added to the blank. Using the following equation, the radical scavenging activity was calculated.

Percentage of Free radical scavenging activity = [(A0 – A) /A0] × 100

Where A0 represents the absorbance of the control solution, which contains all of the reagents other than plant extract, and A represents the absorbance of the DPPH solution including plant extract.

The plant extract concentration (µg/ml) was plotted against the DPPH radical-scavenging activity (%) to estimate the IC50, or the concentration of extract required to reduce DPPH radical-scavenging by 50%. Using SigmaPlot 9 2000 Demo [SPSS Inc., Chicago, IL, USA] and sigmoid non-linear regression, the extract's IC50 value was calculated. Each determination was made three times.

Statistical analysis

Statistics were presented as means and standard deviations. The Student's t test was used for the statistical analysis. P0.05 was used to determine whether differences were significant. Plotting the proportion of inhibition vs concentration yielded the statistical program's (X=Y-C/M) prism dose-response curve, from which the inhibitory concentration 50% (IC50) was derived.

Molecular docking analyses

The PubChem web service was used to determine the phytochemical components of *R. sativus* in order to pick the ligands for the molecular docking investigation [12].

The docking of the target protein Cytochrome-P450 and the ligand phytochemicals and gallic acid was programmed using the CB-Dock (Cavity-detection guided Blind Docking) server [12, 13]. This server predicts the binding sites of a given protein and calculates the centers and sizes using a novel curvature-based cavity detection approach. In order to obtain a success rate of more than 70% in the models created, the server collaborates with AutoDock Vina and has been meticulously optimized. Five potential coupling cavities were found during the studies using the protein files in.pdb format and the ligand in.sdf format. Based on the lowest Vina value found, the candidate with the lowest binding energy was chosen. The ligands and the proteins were then visualized with the space fill and secondary structure respectively.

Figure 1: Harvesting of *R sativus* microgreens

RESULTS AND DISCUSSION

Production and harvest of microgreens

Microgreens are commonly used as food plants throughout their early development. It began to sprout on day 2, and the micro-greens expanded steadily until they reached a height of 4-5 inches on day 8 (Fig. 1). 180 g of microgeens were harvested from 40 g of seeds.

Phytochemical compounds of microgreen and mature *R.sativus* leaf

Sample of dry and wet methanolic extract shows the presence of Alkaloids, flavonoids, terpenoids, polyphenols, and anthocyanins.

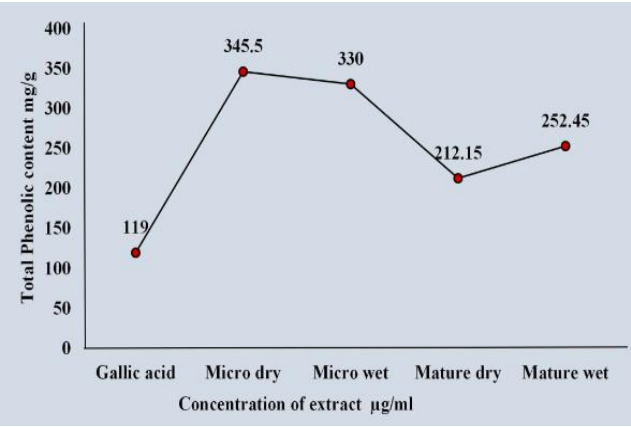
When comparing *R. sativus* mature leaves that are dry an d wet to microgreen methanolic extracts, phytochemicals are more plentiful in dry microgreen leaves, moderately p resent in wet micro greens. This shows that *R. sativus* microgreens have higher amounts of phenolic content than mature plants. Roghini and K. Vijayalakshmi [9] discovered that *R. sativus* roots have lower flavonoid contents and antioxidant capability than *R. sativus* leaves.

ANTIOXIDANT ACTIVITY

Total Phenolic Content - FC METHOD:

In a methanolic leaf extract of *R. sativus,* the total phenolic content was calculated using the Folin-Ciocalteu method. The equation derived from a standard gallic acid calibration curve (Y= 0.7393x+0.481, R=1 119.0 mg/g) was used to calculate the total phenol content as mg of gallic acid equivalent per gram.

The methanolic crude extract of microdry has a significant level of total phenolic content (345.5 mg/g of gallic acid equivalent). Microgreen dry content was found to be 345.5 mg/g, microgreen wet content to be 330.0 mg/g, mature leaf dry content to be 252.45 mg/g, and mature green wet content to be 212.15 mg/g (Graph-1).



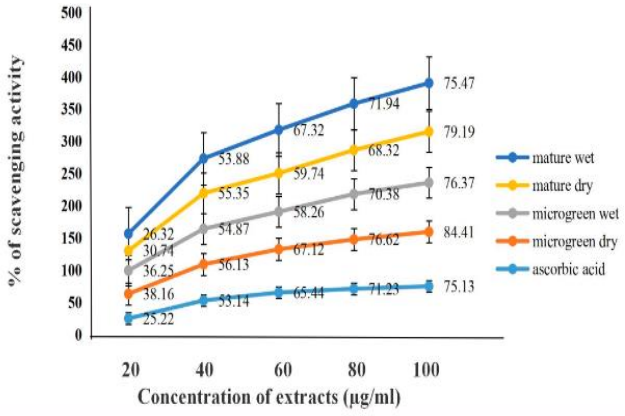
Graph 1: Total Phenolic content of methanolic extract of *R. sativus* leaf

Based on the findings of this study, it is hypothesized that phenolic compounds are significant plant ingredients with redox characteristics that are in charge of antioxidant action. In addition, methanol is the best solvent for extracting polyphenol compounds because it may prevent the polyphenol oxidase process that results in the oxidation of phenolic compounds and evaporates more quickly than water [14, 15].

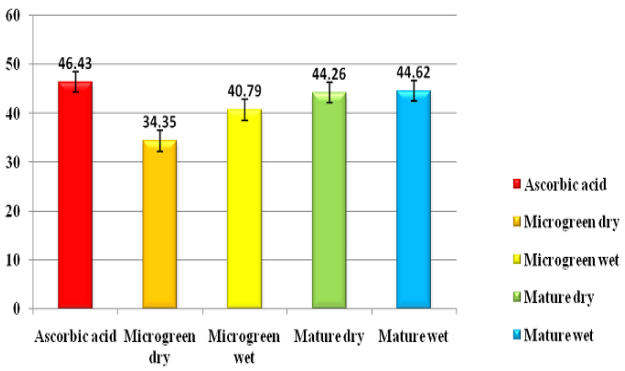
DPPH Free radical scavenging activity:

According to the findings of this study, methanolic extracts of *R. sativus* showed considerable antioxidant activity when tested using the DPPH method. The *R. sativus* microgreen dry sample has stronger antioxidant activity (84.41 g/ml) than the benchmark ascorbic acid at the greatest concentration (100 g/ml) (75.13 g/ml), as shown in **Graph 2**. Furthermore, the methanolic extract of microgreen wet, mature leaf dry, and mature leaf wet all demonstrate notable antioxidant activity (76.37, 79.19, and 75.47 g/ml, respectively).

The Half maximal inhibitory concentration (IC50) value was derived from the calibration graph (y = 0.5895x+22.659=0.8572) of standard ascorbic acid was found to be **46.43463** µg/ml compared to this the methanolic extract of *R.sativus* microgreen dry leaf and mature dry leaf exhibited high antioxidant activity (**34.35398 and 44.2623**µg/ml) but the microgreen wet leaf and mature leaf wet sample were not so effective (**40.79498 and 44.62995**µg/ml) was lower than the standard (**Graph 3**).



Graph 2: Radical scavenging activity of methanolic extract of *R. sativus* leaf



Graph 3: IC_{50} value of standard ascorbic acid and methanolic extract of *R.sativus leaf*

By contrast with its non-edible parts, which leaves are as effective as peels in the anti-bacterial, analgesic, acute,

and chronic anti-inflammatory effects, Gheith, I., and El-Mahmoudy (2017) revealed in vitro and in vivo have recorded the superior antioxidant capacity of pomegranate leaves. These leaves to suppress hydroxyl radicals were demonstrated by authors to be greater than that of the flowers [16, 17].

IN SILICO STUDIES

Raphanus sativus's phytochemicals were chosen for this their structures were taken from the pubchem database [12] for the in silico investigation, which was then followed by the creation of the ligand. The Lipinski rule of five and pharmacokinetics characteristics were used to examine the ligands. CB Dock software (Cavity-detection guided Blind Docking) server [13] was used to analyze the docking of ligand molecules for antioxidant activity with the target protein Cytochrome P450. The results were contrasted against a positive control, Gallic acid.

Target protein:

Human cytochrome P450 (CYP2C9) was chosen as the target protein in this study.This sequence was looked up in the PDB database [https://www.rcsb.org/], and the FASTA sequence was obtained. The pdb format for the target protein was created using the Swiss model database. Figure 2(a) displays the structure and Swiss model data identified, while Figure 2(b) lists the Z-score.

From the Z-score, GMQE value was 0.91, QMEAN value was 0.07, solvation value was 1.01, torsion value was -0.32. higher QMEAN Z score values means better agreement with the predicted feature [19].

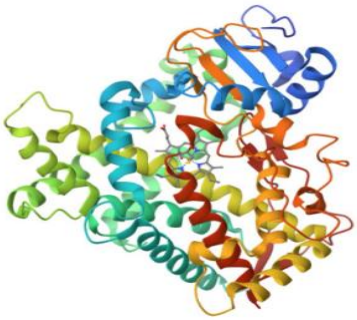


Figure 2a: 3D structure of Cytochrome P450

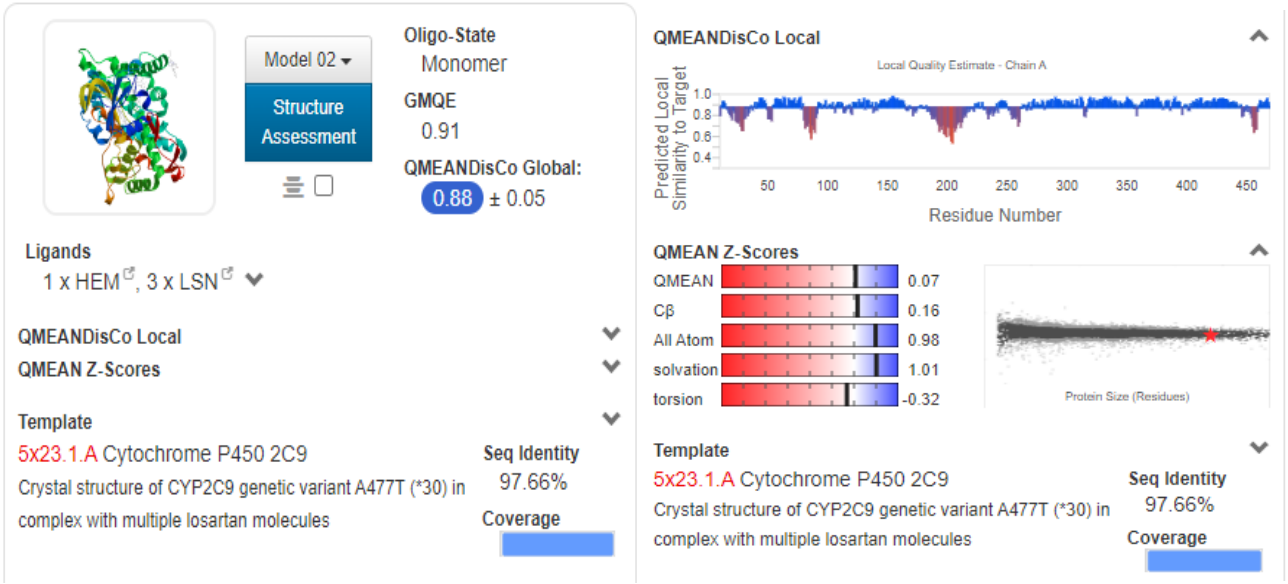


Figure 2b: Z-score value for Cytochrome P450

Table 1: Molecular binding affinity of the target protein (10G2) with the ligand molecule

Sl.No	Target protein	Ligand	Cavity size	Binding score
1	Cytochrome P450 (1OG2)	Gallic acid	2391	-6
2		Epicatechin	3461	-8.4
3		2,3-dimethylphenol	2391	-5.6
4		Quercetin	2391	-8.9
5		2-methoxy-4-methoxyphenol	2391	-5.6
6		Vanillic acid	3461	-5.6
7		p-coumaric acid	2391	-6.4
8		3-hydroxy-beta-ionone	2391	-7.0

Molecular docking:

Using CB dock, the chosen phytochemicals epicatechin, 2,3-dimethylphenol, quercetin, 2-methoxy-4-methoxyphenol, Vanillic acid, p-coumaric acid, 3-hydroxy-beta-ionone, and positive control gallic acid were docked against the target protein, cytochrome P450 (10G2) [20]. The computations have been completed, and the results are shown in the Table 2 below along with the binding affinity (kcal/mol) values. The ligand will be better positioned at the binding site the more negative the binding affinity.

The binding affinity of the target protein and the ligands is represented by the docking score (Table 1). This finding shows that the phytochemicals quercetin (-8.9), epicatechin (-8.4), 3-hydroxy-beta-ionone (-7.0), and P-coumaric acid (-6.4) have greater efficacy with cytochrome p450 than gallic acid (-6.0) (Fig 3).

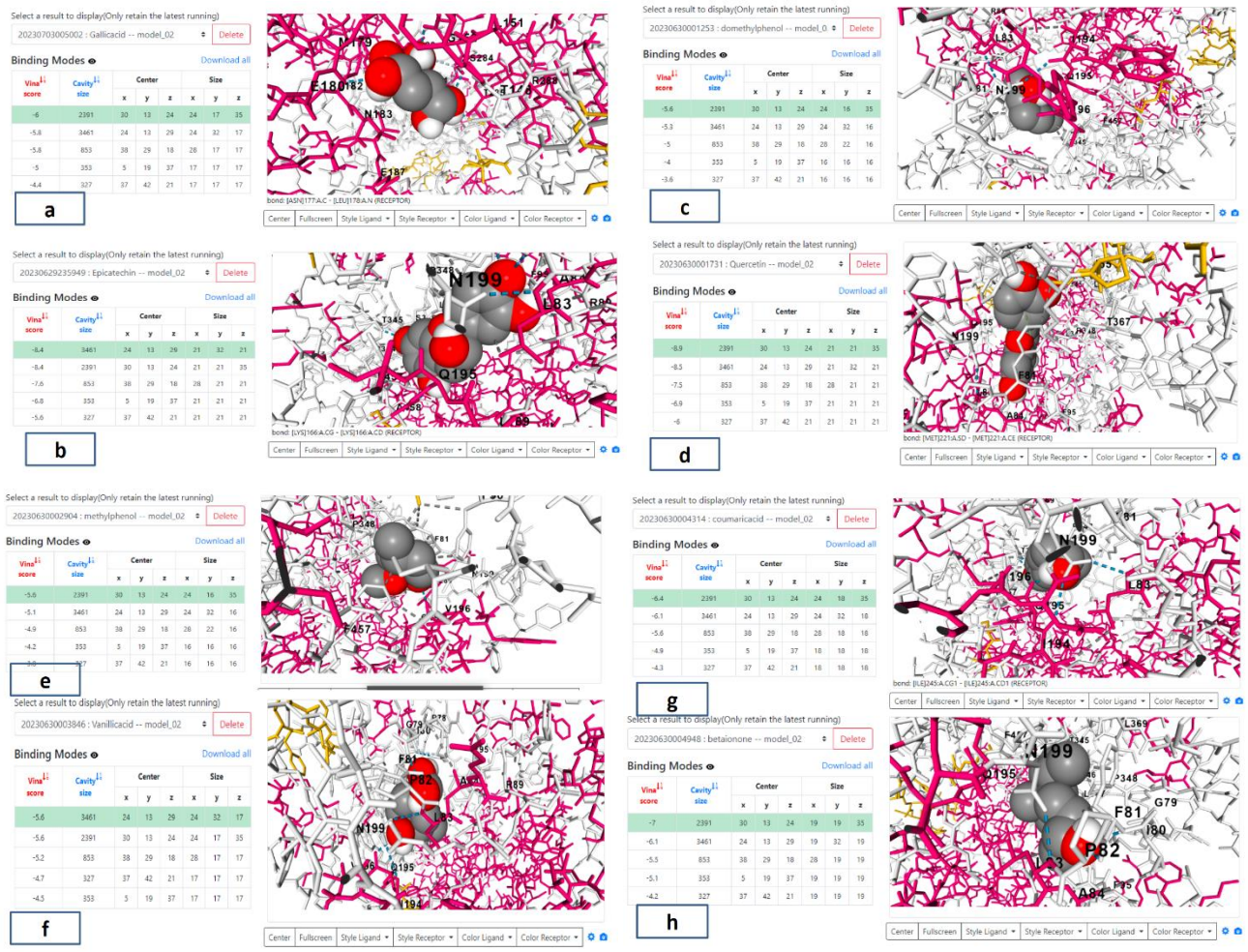


Figure 3: Docking scores and docked amino acid residues of phytochemicals with target protein Cytochrome P450

In the docked amino acid residues - PHE-134 amino acids are interacted in 2-methoxy-4-methoxyphenol, vanillic acid, 2,3-dimethylphenol and amino acids are interacted in phytochemical components including gallic acid, GLU-325 amino acids are interact in Epicatechin and 3-hydroxy-beta-ionone, ASN-133 amino acids are interact in vanillic acid, 2,3-dimethylphenol and 3-hydroxy-beta-ionone, which represents their activity in particular protein-ligand interaction.

This study demonstrated that phytochemicals are more effective than the positive control, and it is recommended that this be further investigated in more sophisticated methodologies and clinical trials.

CONCLUSION

The current study mainly concentrated on the chemical components of *Raphanus sativus* microgreen and mature leaf extracts, which have several bioactivities. Gallic acid has 119 mg/g of total phenolic content, whereas microgreen dry extract has 345.5 mg/g. A standard gallic acid equivalent of mg per gram was used to calculate the total phenolic content. Given that *R. sativus* contains a high concentration of phenolic compounds, these compounds may play a significant role in this plant's antioxidant activity. The IC_{50} value of the microgreen dry extract was 34.35398 g/ml, which was higher than the ascorbic acid (standard) value of 46.43463 g/ml, indicating adequate activity. This study found that the methanolic extract of *R sativus* has a significant total phenolic content and antioxidant activity.

From this docking investigation, it is hypothesized that the phytochemicals quercetin, epicatechin, 3-hydroxy-beta-ionone, and P-coumaric acid have greater effectiveness against cytochrome P450. This shows that these elements were taken into consideration while choosing the prodrug

formulation. *R. sativus* microgreens are regarded as a potent source of dietary nutrients. It does, however, have beneficial biological and therapeutic properties. Based on these findings, it was hypothesized that, in the future, attention should be given to the creation of medications using phytochemicals rather than man-made chemical components because they are said to be more affordable, readily available, less poisonous, and have less adverse effects. For further validation, in-vivo and preclinical testing can be used to study this further.

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