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RESEARCH ARTICLE

In vitro and *In Silico* Study of Anti Diabetic Activity on *Raphanus sativus* Microgreen and Mature Leaf

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ABSTRACT

Diabetes is one of the biggest issues the world is currently facing. The leaves of *Raphanus sativus* methanolic extract were used to screen phytochemicals and antidiabetic effects (by using alpha-amylase inhibition assay). To acquire their crude extracts, the *R. sativus* leaves were powdered and macerated in methanol after being sun-dried. The percentages of inhibition for the methanolic extracts of *R. sativus* were determined to be 79.34%, 78.45%, 77.62%, and 74.74%, (respectively), for the microgreen dry, microgreen wet, mature green wet, and mature green dry. Similar results were reported for the inhibitory concentration–50 values of these methanolic extract. When compared to other methanolic extracts, the dry methanolic extract of *R. sativus* microgreens demonstrated higher potency. In addition, *in silico* analysis was performed using the using hex 8.0.0 software. Through this identified the binding score of the phytochemical component with alpha amylase enzyme.

Keywords: Anti diabetic activity, Alpha amylase inhibition activity, *Raphanus sativus*, Microgreen

INTRODUCTION

Notably, diabetes mellitus is one of the world's most prevalent metabolic illnesses that are associated by lipid and carbohydrate digestion [Joshi S R *et al*., 2015].[1] One of the leading causes of human mortality, diabetes has become a major health risk on a global scale [Dal-Ré, R, 2011].[2] Due to the fact that most common medications for treating diabetes require regular injections, the duration of the treatment is long. Even, what we utilizing antidiabetic therapies are not a single-dose program. Although several common medications have been identified for their ineffectiveness and severe side effects [Gupta P *et al*., 2016].[3] Therefore, further research is still needed into effective oral medicines or common household cures for controlling diabetes.

Synthetic hypoglycemic drugs are typically a double-edged sword and may cause harmful complications such as gastrointestinal irritation, nausea, and thyrotropin suppression, the World Health Organization expert committee recommended investigating and taking into consideration antidiabetic agents of plant origins. Natural remedies with a plant origin are well known for serving as both curative and preventive agents, as well as having lower toxicity and adverse effects.

The Radish comes under the family of *Brassicaceae*, which has over 310 genera and 3500 species [OECD 2016][4], is one of the most regularly consumed vegetable. It has specific properties that result from the presence of phytochemicals; it has a low caloric count, rich source of calcium, magnesium, copper, manganese, potassium, Vitamin B6, Vitamin C, and folate (Gupta R *et al*., 2003).[5] Researchers and the pharmaceutical sector are now interested

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in the potential use of *Raphanus sativus* as a source of bioactive chemicals that may have therapeutic and health implications in conditions including hypertension, cardiometabolic disorders, antioxidants, and antibacterial agents [Manivannan A *et al*., 2019].[6] To better understand the role that *R. sativus* plays in human nutrition and health, as well as to guide future biological and pharmacological research on the wide spectrum of phytochemicals that are present in this vegetable, a thorough and systematic evaluation of the nutritional and phytochemical composition of this plant is necessary [Manivannan A *et al*., 2019].[6]

Microgreens are young and edible greens that are between sprouts and baby greens in size and are typically picked 7–21 days after germination, when the plant's first genuine leaves have appeared. The fact that microgreens may be grown outdoors, in greenhouses, and even on a windowsill makes them very convenient to grow. A variety of seeds can be used to grow microgreens.

Brassicaceae,*Asteraceae*,*Apiaceae*,*Amaryllidaceae*, *Amaranthaceae*, and *Cucurbitaceae* are the plant families whose seeds are used to create the most popular variants. According to studies comparing microgreens to mature greens, the amount of nutrients in microgreens can be up to 9 times more than those in mature greens.

The generation of microgreens is therefore planned as part of the overall investigation to reveal the phytochemical components. *R. sativus* microgreen, mature leaves, and *in silico* analyses were used to examine the mechanism of action inhibition.

MATERIALS AND METHODS

Microgreen production

The seeds (*R. sativus* seeds) required to produce microgreens are purchased from Shilpa Hi-tech Seeds, Bangalore. The trays were filled with soil, the seeds were scattered on top of the soil as evenly as possible, and they were lightly misted with water twice a day, until the 8th day the growth was observed.

Sample collection

Microgreen (in trays) leaves and mature leaves (collected from local markets) after it used for wet sample extraction. Water was used to clean, rinse, and collect the samples. To get a dry sample, the leaves were left to dry in the shade of the sun and made powder and its utilized for dry extraction.

Extraction

Methanol is combined with leaf powder (microgreen and mature 20 g each) to create a fine paste (1:10 ratio). The sample was obtained and held to allow the methanol to evaporate using a sonicator (24 h). After being collected, the extract was centrifuged at 5000 rpm for 10 min. The pure extracted samples were collected and placed in a conical flask with aluminum foil on top [Roghini R and Vijayalakshmi V, 2018], this extract is used for further analysis.[7]

Qualitative analysis of phytochemical compounds

Based on Radha *et al*. study's from the year 2021, the phytochemicals underwent a qualitative examination.[8]

In vitro study

Alpha-amylase inhibitory assay

The alpha-amylase assay was carried out with some modifications to the procedure outlined by [Kwon YI *et al*., 2007].[9] Test tubes were filled with varying amounts of plant extract (20–100 μg/mL) that had been diluted in phosphate buffer, along with alpha-amylase enzyme in various concentrations. The reaction was started by adding 250 μL of a 1% starch solution after the initial 10 min of incubation at 25°C. Following the addition of 500 μL of DNS reagent to each test tube, the reaction was then stopped by incubating the test tubes for 5 min at 100°C before cooling to room temperature. After that, 5 mL of distilled water was added to each test tube to dilute them all. A sample without alpha-amylase enzyme is called a "blank." As a positive control, acarbose (20–100 μg/mL) was employed in place of the extract.

$$\%inhibition = \frac{Ab(control) - Ab(test)}{Ab(control)} \times 100$$

Determination of inhibitory concentration–50 (IC_{50}) for *in vitro* α-amylase inhibitory activity

The importance of calculating the extract solution's IC_{50} is related to a comparison of the potency of two substances. The lower the IC_{50} number, the more effective the compound is. For instance, two compounds may exhibit nearly identical levels of inhibition at a given dose. To distinguish one compound from another in terms of effectiveness, the IC_{50} value is calculated.

Statistical analysis

Statistics Information was presented as means SD. The Student's t test was used for the statistical analysis. At $P = 0.05$, differences were deemed significant. The Prism dose-response curve (statistical programme) was used to calculate the IC_{50}, which was derived by graphing the percentage of inhibition versus concentrations.

In silico study

In this *in silico* work, the Pubchem database [https://pubchem.ncbi.nlm.nih.gov/] was used to identify the phytochemical components of *R. sativus* structures.[10] The ligand is prepared by making sure the atoms are assigned in the proper order. Before moving on to docking, it is should have to check the volation (zero violations), adhere to the Lipinski Rule of Five [https://www.molinspiration.com],[11] and exhibit good pharmacokinetic activity [http://www.swissadme.ch/].[12] Cheminformatic techniques for the online smile translator [https://cactus.nci.nih.gov/] were used to assemble ligand molecules from seven phytochemical components from *R. sativus*. This process transforms the Smiles Strings into a pdb format file. Pymol software was used to visualize the resultant ligand.[13]

RESULTS AND DISCUSSION

Production and harvest of microgreen

In its early stages of growth, microgreens are typically used as an edible plant. On June 8, 2022, *R. sativus* seeds were seeded on mud, sprouting started on day 2, and gradually the micro-greens were grown up to 4–5 inches each day till day 8 [Figure 1]. On June 16, 2022, the grown microgreens were carefully collected with scissors. 150 g of micro-greens were gathered and the extraction process was started.

Phytochemical compounds of *R. sativus* leaf

From this study's, methanolic extract of dry microgreen *R. sativus* [Table 1] provided evidence of the presence of alkaloids, flavonoids, steroid, tannin, terpenoids, polyphenolics, saponins, coumarin, anthocyanins, and leucoanthocyanins.

The phytochemicals in *R. sativus* are most abundant in the dry microgreen leaves, slightly present in the wet microgreen and dry mature leaves, and less abundant was found in the wet mature leaves. This shows that the phytochemical content of microgreen *R. sativus* is higher than that of mature ones. Hence, according to Roghini R and Vijayalakshmi V (2018), this root was thought to be less healthy for humans than its leaves.[7]

Anti diabetic activity

The findings of this study showed that microgreens of *R. sativus* had a substantial impact on alpha-amylase. The standard (acarbose) showed the effect of alpha-amylase by 58.76% at the maximum concentration (100 µg/mL) examined. The methanolic extract of *R. sativus*, however, showed 79.34% for the microgreen dry sample, 78.45% for the microgreen wet sample, 74.74% for the mature green dry sample, and 77.62% for the mature wet sample [Graph 1]. Hence, compared to the other extract and the control, the microgreen dry extract exhibits a notable outcome.

The IC_{50} value for standard acarbose in this investigation was determined to be 84.79263 g/mL using the regression graph [$y = 0.4346\times + 13.202$ $R2 = 0.9886$]. The microgreen dry leaf extract's IC_{50} values of 37.08904 g/mL were superior to those of the other extracts and the industry standard. Table 2 displays the regression and IC_{50} values.

Following the hydrolysis of glycosidic linkages in digestible carbohydrate diets containing starch by

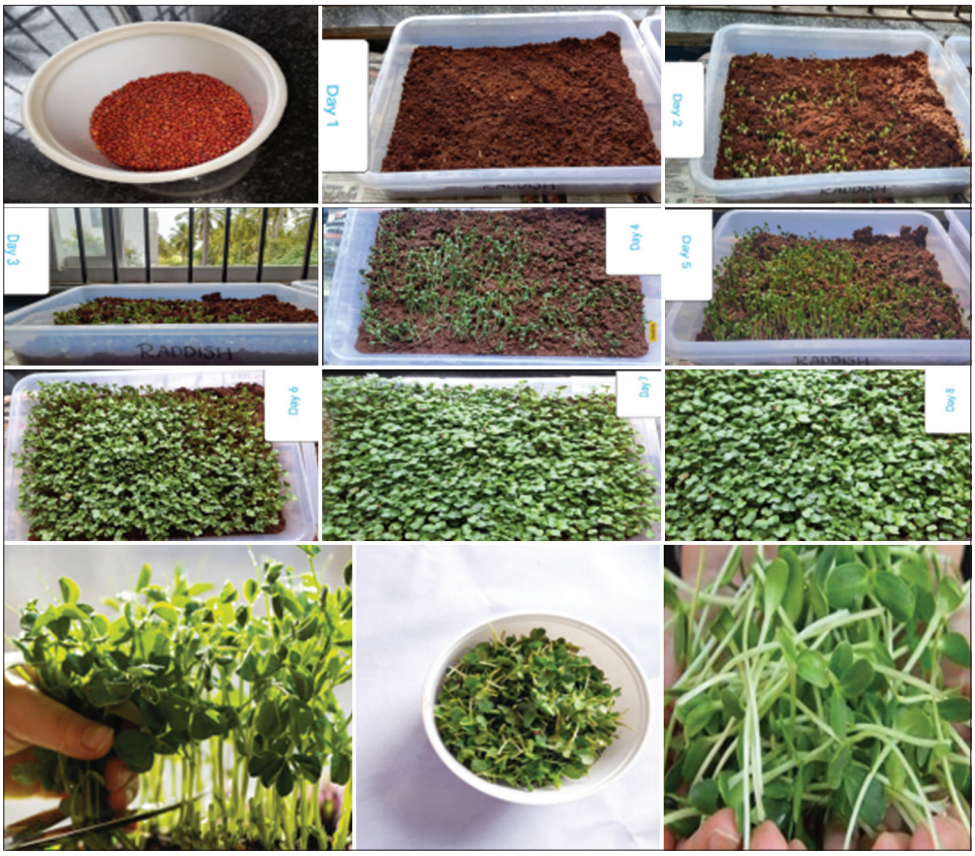
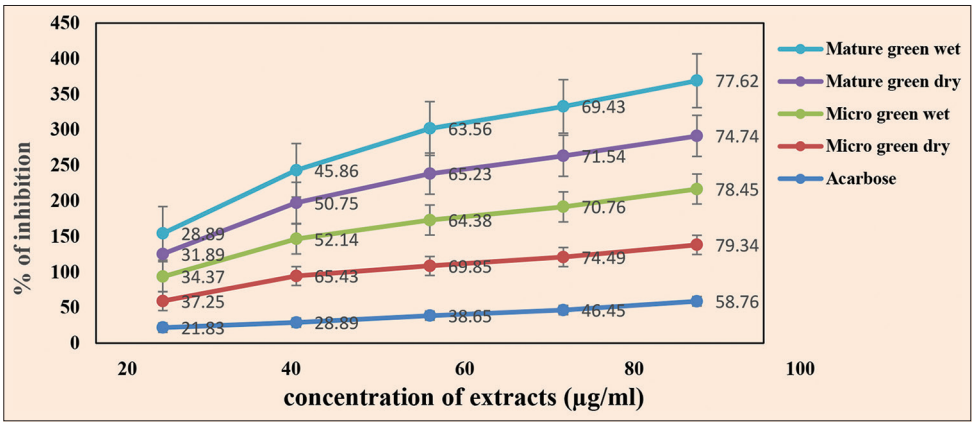


Figure 1: Production and harvest of microgreen



Graph 1: % of inhibition of Alpha amylase inhibitory activity of acarbose and *Raphanus sativus* leaf methanolic extract

the enzyme alpha-amylase, glucose can be easily absorbed from the gastrointestinal system into the bloodstream. An alpha-amylase inhibition *in vitro* screening was utilized to examine the potential hypoglycemic effects of the methanolic extract of *R. sativus* [Conforti F *et al*., 2005]. Inhibition of these enzymes lowered the high postprandial blood glucose peaks in diabetics.[14]

Tamil IG *et al*. (2010) noted that the hexane extract of P. amarus, the N-hexane extract of *Amaranthus caudatus* var. *Oscarblanco*, and the ethyl acetate extract all had high IC_{50} values. As a result, the methanolic extract of *R. sativus* microgreen dry exhibits a better IC_{50} value (37.08904), followed by microgreen wet (43.3978). More phytochemical components such flavonoids, alkaloids, and phenolic compounds may be the cause.[15]

The plant-based alpha-amylase inhibitor provides a potential therapeutic method for the management of diabetes in the ethanol, methanol, and hexane

Table: 1 Phytochemical compounds of microgreen and mature *Raphanus sativus* leaves

Compounds	Dry microgreen	Dry mature leaves	Wet microgreen	Wet mature leaves
Tannins	+++	++	+	+
Flavonoids	+++	+++	+++	++
Terpenoids	+++	+++	+++	++
Saponins	++	+	+	+
Alkaloids	+++	+++	+++	++
Phenolics	+++	++	+++	++
Steroids	++	+	+	+
Anthocyanin	+++	+++	+++	++
Leuco anthocyanins	++	++	++	+
Coumarins	++	++	+	+
Reducing sugar	++	++	++	+

Table 2: IC_{50} values of *Raphanus sativus* alpha-amylase inhibitory activity

Samples	X (IC_{50})	Y	C	M
Acarbose	84.79263	50	13.2	0.434
Microgreen dry	37.08904	50	28.34	0.584
Microgreen wet	43.39789	50	25.35	0.568
Mature green dry	47.79772	50	20.7	0.613
Mature green wet	51.39706	50	15.05	0.68

extracts [Conforti F *et al*., 2005]. According to this study, when compared to the commonly used anti-diabetic medicine Acarbose, the methanolic leaf extract of the microgreen dry *R. sativus* demonstrated significant alpha-amylase inhibitory properties.[14]

Molecular docking

The hex 8.0.0 tool was used to dock the selected phytochemicals epicatechin, 2,4-dimethylphenol, quercetin, 2-methoxy-4-methylphenol, vanillic acid, p-coumaric acid, and 3-hydroxy-beta-ionone against the target protein alpha-amylase. Pymol software was used to obtain the target protein that was bound with the ligand and the appropriate amino acid residues. Table 3 lists the docking scores for amino acid residues. Figure 2 depicts the docked ligand-protein structures.

From this docking study, it indicates ARG 398, 267 have good interaction with Epicatechin, 2,4-dimethylphenol, and 2-methoxy-4-methylphenol component. GLY 403,334 have an interaction with Epicatechin, and 3-hydroxy-beta-ionone. HIS 305, 299 have an interaction with P-coumaric acid. From this it speculated that, *R. sativa* phytochemical component have better affinity with acidic and basic group of amino acids. Hence it is demonstrated that phytochemicals are more efficient and it is

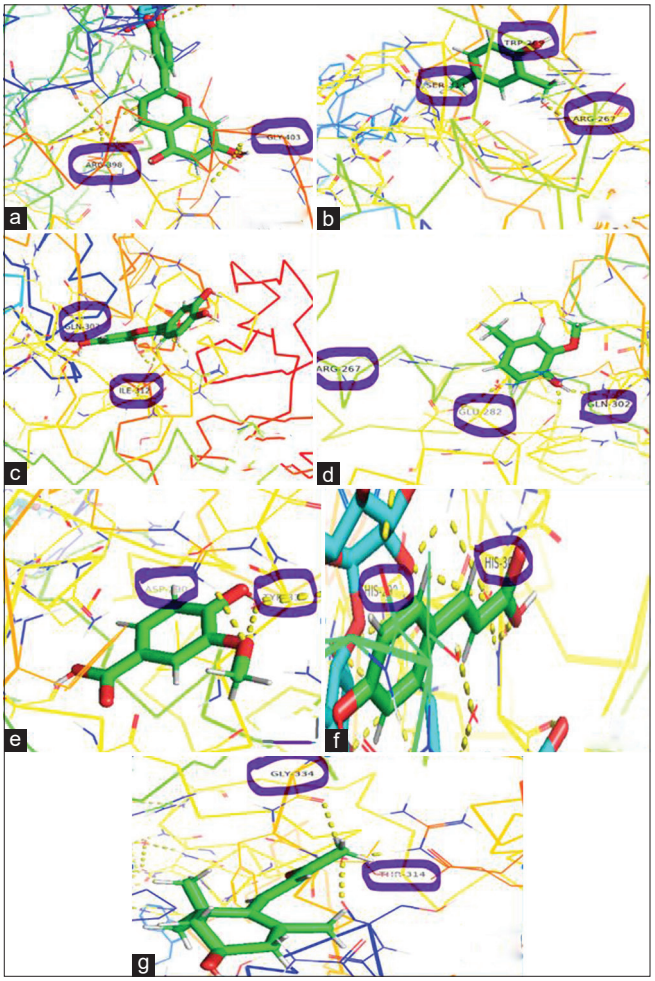


Figure 2: Docked amino acid residues phytochemical component with Alpha amylase. (a) Epicatechin, (b) 2,4-dimethylphenol, (c) Quercetin, (d) 2-methoxy-4-methylphenol, (e) Vanillic acid, (f) P-coumaric acid, and (g) 3-hydroxy-beta-ionone)

Table 3: docking scores and docked amino acid residues of phytochemicals with target protein alpha-amylase

Ligands	Target protein	Docking score [-KJ/Mol]	Number of docked amino acids	Amino acid residues
Epicatechin	Alpha-amylase	−lpha-amylases	2	ARG-398 GLY-403
2,4-dimethylphenol		−,4-dimethylph	3	SER-311 ARG-267 TRP-269
Quercetin		−uercetinhylph	2	GLN-302 ILE-312
2-methoxy-4-methylphenol		−-methoxy-4-me	3	ARG-267 GLU-282 GLN-302
Vanillic acid		−acidlic acide	2	ASP 290 TYR 333
P-coumaric acid		−acidric acide	2	HIS-305 HIS-299
3-hydroxy-beta-ionone		−-hydroxy-beta	2	GLY-334 THR-314

recommended that *in vivo* methods and clinical trials be used to further examine their effectiveness.

CONCLUSION

The high nutrient content of microgreens makes them very popular in modern society. Microgreens of high quality and quantity were grown as a result of this investigation. The effect of microgreens is superior to that of mature *R. sativus* leaves. The primary goal of this study is to identify the numerous chemical components of *R. sativus*. According to this *in vitro* study, dry microgreen *R. sativus* may had a significant alpha-amylase inhibitory impact. To comprehend the antidiabetic activity and phytochemical components for the future drug research, it may be interesting to study the positive effects of *R. sativus* components. To examine the effectiveness of phytochemicals as a ligand in the inhibition of the target protein alpha-amylase, an *in silico* analysis of *R. sativus* was undertaken.
In light of this, these phytochemicals may be used in prodrug formulation. As a result, I draw the conclusion that in the future, attention should be paid to creating pharmaceuticals using phytochemicals rather than synthetic chemical components because they are said to be less toxic, cheaper, more readily available, and associated with less side effects. For additional validation, this can be looked into more thoroughly through *in vivo* and preclinical testing.

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DISCLOSURE STATEMENT

Nil.

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