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# Identification of *Macrolepiota procera* extract as a novel G6PD inhibitor for the treatment of lung cancer

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## ABSTRACT

Tumor metabolism, an emerging hallmark of cancer, is characterized by aberrant expression of enzymes from various metabolic pathways including glycolysis and PPP (pentose phosphate pathway). Glucose 6 phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD), oxidative carboxylases of PPP, have been reported to accomplish different biosynthetic and energy requirements of cancer cells. G6PD and 6PGD have been proposed as potential therapeutic targets for cancer therapy during recent years due to their overexpression in various cancers. Here, we have employed enzymatic assay based screening using in-house G6PD and 6PGD assay protocols for the identification of mushroom extracts which could inhibit G6PD or 6PGD enzymatic activity for implications in cancer therapy. For the fulfillment of the objectives of present study, nine edible mushrooms were subjected to green extraction for preparation of ethanolic extracts. 6xhis-G6PD and pET-28a-h6PGD plasmids were expressed in BL21-DE3 *E. coli* cells for the expression and purification of protein of interests. Using purified proteins, in house enzymatic assay protocols were established. The preliminary screening identified two extracts (*Macrolepiota procera* and *Terfezia boudieri*) as potent and selective G6PD inhibitors, while no extract was found highly active against 6PGD. Further, evaluation of anticancer potential of mushroom extracts against lung cancer cells revealed *Macrolepiota procera* as potential inhibitor of cancer cell proliferation with IC<sub>50</sub> value of 6.18 µg/ml. Finally, screening of *M. procera*-derived compounds against G6PD via molecular docking has identified paraben, quercetin and syringic acid as virtual hit compounds possessing good binding affinity with G6PD. The result of present study provides novel findings for possible mechanism of action of *M. procera* extract against A549 via G6PD inhibition suggesting that *M. procera* might be of therapeutic interest for lung cancer treatment.

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## 1. Introduction

Lung cancer is the most common cancer in males and second most prevalent cancer among females after breast cancer (Majeed et al., 2019). Irrespective of the advances in understanding the causes and risks of lung cancer, it remains the leading cause of deaths worldwide (Bade and Dela Cruz, 2020). Thus, there is an urgent need for the development of potent and selective therapeutics against lung cancer.

Cancer cells reprogram their metabolic pathways to fulfil their energy requirements and anabolic demands (Enzo et al., 2015). There are various rewired metabolic pathways in tumor cells, among these, pentose phosphate pathway (PPP) plays a critical role

in cancer progression (Vazquez et al., 2016). PPP fuels cancer cells by producing ribonucleotides and phosphopentoses, acts as a chief source of NADPH and plays key role in maintaining cellular redox state in tumor cells (Dang, 2018). G6P is a first rate limiting enzyme of PPP which plays various significant roles such as, redox balance, lipid synthesis, generation of nucleotide precursors and maintenance of cellular metabolic homeostasis (Zmijewski, 2019). Numerous studies have reported critical role of G6PD in survival, proliferation and metastasis of cancer cells (Nakamura et al., 2020). 6-phosphogluconate dehydrogenase (6PGD) is an oxidative carboxylase of PPP which is responsible for the conversion of 6-phosphogluconate into ribulose-5-phosphate in presence of NADP. Upregulation of G6PD and 6PGD has been reported in various human cancers such as ovarian, acute myeloid leukemia, breast and lung cancer (Cho et al., 2018). Thus, targeting G6PD and 6PGD to deprive cancer cells presents a promising approach to combat cancer.

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Given that both enzymes, G6PD and 6PGD, are emerging targets for cancer treatment (Zhang et al., 2019; Yang et al., 2018), it is of great interest to find out inhibitors of these enzymes from natural sources. Mushrooms have long traditional history of medicinal use in Asian region (Jeitler et al., 2020). Many research studies have reported the promising ability of mushrooms to target various deregulated pathways of tumor cells. Therefore, there is growing interest in mushrooms in the recent years for the development of novel therapies against cancer (Hetland et al., 2020). Thus, the aim of present study was screening of ethanolic mushroom extracts for the identification of G6PD and 6PGD inhibitors.

## 2. Materials and methods

### 2.1. Collection and identification of mushrooms

The mushrooms were collected from various regions of Osmaniye, Turkey. After collection, their identification was done. The list of collected mushrooms is provided in Table 1.

### 2.2. Preparation of mushroom extract library

After collection and identification, mushrooms were washed by water and air dried at a shady place. After that, the dried mushrooms were grinded in order to obtain a fine coarse powder. As a next step, the mushroom extracts were prepared by using green extraction technology. For this purpose 200 g of coarse powder material was taken in a beaker and 600 ml (80%) ethanol was added in it. The mixture was heated at the power of 400 W for 6 min in a microwave oven. After completion of heat cycles, the mixture was filtered and the filtrate was subjected to rotary apparatus followed by the lyophilization of crude extracts for 2–3 days. These extracts were stores at 4 °C for further analysis.

### 2.3. Expression and purification of proteins

#### 2.3.1. G6PD expression and purification

G6PD plasmid was gifted by a German physician and biochemist Prof. Dr. Katja Becker. The sequence of G6PD was obtained from (GenBank accession No. NP-001035810). The plasmid was cloned into pET24a (Novagen). To perform overexpression, 2xYT medium was prepared with a composition of; 10 g yeast,

50 µg/ml kanamycin, 16 g tryptone, 5 g NaCl/ liter and 12.5 µg/ml chloramphenicol. During overexpression, the temperature was kept constant at 23 °C. IPTG (0.1 mM) was used to induce protein expression at an optical density of 600 nm. After 24-hours, cells were collected, lysed and purified. For the purpose of purification, metal affinity chromatography was performed using Ni-NTA column with 300 mM NADP<sup>+</sup> and final pH of 8.0. After purification, G6PD was rinsed from Ni-NTA column by using 300-mM imidazole. The purified G6PD protein was stored at 4 °C temperature in a medium containing ammonium sulfate (1.8 M) and NADP<sup>+</sup> (0.1 mM) (Preuss et al., 2012).

#### 2.3.2. 6PGD expression and purification

For the expression and purification of His-tagged protein of interest, 6PGD, cells were lysed in 3 to 5 ml of lysis buffer per gram of cells and after that they were subjected to sonication in order to agitate the particles in buffer solution. The lysate solution was subjected to cold temperature in order to prevent any proteolysis. After that, the lysate solution was centrifuged for 30 min at 13000 rpm and 4 °C temperature. To bind His-tagged protein, 50% Ni<sup>2+</sup>-NTA beads solution was added. In the next step, slurry of Ni<sup>2+</sup>-NTA was loaded in the proteins of the column, and the column was subjected to washing by buffer. After that, the elution of column was done with imidazole. SDS-PAGE was performed for protein purification (Braun et al., 2002).

### 2.4. Establishment of enzymatic assays

#### 2.4.1. G6PD enzymatic assay

In order to find out the inhibitors, purified G6PD was subjected to enzymatic activity assay. Activity of G6PD was detected by increasing optical density at 340 nm due to generation of NADPH. As the measurement of absorbance at 340 nm on a multi-well plate was incompatible, hence, a modified colorimetric assay for G6PD activity was developed.

#### 2.4.2. 6PGD enzymatic assay

Enzymatic activity assay was performed of purified 6PGD in order to find out inhibitors. The activity of 6PGD depends upon the production of NADPH and this process was observed by an increase in optical density at 340 nm. A modified colorimetric assay was developed for the measurement of 6PGD activity, which

**Table 1**

List of collected mushrooms from Turkey along with the common and scientific names.

Sr. No.	Library no.	Scientific name	Common name	Images
1.	M1	<i>Agaricus arvensis</i>	Horse mushroom	
2.	M2	<i>Inocybe geophylla</i>	White fibercap	
3.	M3	<i>Lactarius deliciosus</i>	Red pine mushroom	
4.	M4	<i>Lepista nuda</i>	Wood blewit	
5.	M5	<i>Macrolepiota procera</i>	Parasol mushroom	
6.	M6	<i>Pleurotus eryngii</i>	Oyster mushroom	
7.	M7	<i>Scillus luteus</i>	Slippery jack	
8.	M8	<i>Terfezia boudieri</i>	Desert truffle	
9.	M9	<i>Tuber aestivum</i>	Summer truffle	

worked on the principle that reaction of 6PGD with its substrates results in generation of NADPH. NADPH on reacting with PMS and NBT produces formazan. In the next step, the absorbance of formazan was measured at 580 nm by using spectrophotometer. (Chan et al., 2013).

## 2.5. Cell culture

Human lung cancer cell line (A549) was grown in DMEM (Dulbecco's Modified Eagle Medium) containing 10% FBS and 100 µl/ml penicillin streptomycin. Cultured cancer cells were then incubated at 37 °C with a continuous supply of 5% CO<sub>2</sub> in a CO<sub>2</sub> incubator (Rasul et al., 2012).

### 2.5.1. MTT cytotoxic assay

The anticancer potential of mushroom extracts was determined by using MTT assay. To culture cancer cells, 96 well plates were used. Cells were seeded in each well. These cells were incubated for overnight and followed by the treatment of mushroom extracts with the concentrations; 25, 50, 75, 100 and 125 µg/ml. Unpolished media was used in negative control and taxol was used as positive control. After 24 h, 10 µl of MTT (5mg/mL) solution was added to each well and incubated for 4 h. In order to obtain the precise results, cells were used in triplicates. After adding 150 µl of DMSO, absorbance was observed by an ELISA reader at 570 nm wavelength.

The absorbance of control cells and treated cells was used to examine the extract cytotoxicity according to given formula:

$$\% = \frac{A570 \text{ (control)} - A570 \text{ (treated)}}{A570 \text{ (control)}} \times 100$$

## 2.6. Docking studies

Molecular docking study was performed to determine the possible binding sites and affinity of compounds that found in the extract. Glucose 6-phosphate dehydrogenase catalyses the conversion of glucose-6-phosphate to gluconolactone 6-phosphate in the presence of NADP<sup>+</sup>. We downloaded the two crystal structures of human glucose 6-phosphate dehydrogenase complexed with structural both NADP<sup>+</sup> (1QKI) and G6P (2BHL) (Au et al., 2000; Kotaka et al., 2005). The 3D conformer of phytochemicals was retrieved from PubChem website in SDF format. The enzyme and compounds was loaded on Molegro Virtual Docker software (Molegro, 2019). The residue errors in the structure of protein were rebuilt. The cavity to which the co-enzyme and substrate are embedded were defined as docking areas. Ten trials were performed for each compound, and the best binding results were evaluated. Critical analysis of compounds was conducted using UCSF Chimera and discovery studio visualizer software (Pettersen et al., 2004).

## 3. Results

### 3.1. In vitro inhibitory effects of ethanolic mushroom extracts against G6PD and 6PGD

Our in house enzymatic assay protocols were used to determine the inhibitory potential of nine extracts of different mushrooms against 6PGD and G6PD. In this preliminary screening, the inhibiting activities of ethanolic mushroom extracts were investigated at standard dose of 50 µg/ml against both enzymes and the obtained results are presented in the Table 2. The highest inhibition (94%) was expressed by the *Macrolepiota procera* extract against G6PD followed by *Terfezia boudieri* (87%). *Agaricus arvensis*, *Lactarius deli-*

*ciosus*, *Pleurotus eryngii*, and *Scillus luteus* extracts exhibited the moderate G6PD inhibitory activity (56%, 54%, 54%, 53% respectively) while three mushroom extracts (*Inocybe geophylla*, *Lepista nuda*, *Tuber aestivum*) were found to be inactive against G6PD. On the other hand, *Tuber aestivum* extract was the most potent against 6PGD with inhibitory activity of 63%, while all other mushroom extracts showed <50% inhibition against 6PGD.

The activities of all extracts against 6PGD and G6PD are summarized in Table 3 which indicates that out of nine extracts, two extracts were highly active against G6PD and while no extract was found highly active against 6PGD. Thus, these findings revealed *Macrolepiota procera* and *Terfezia boudieri* as interesting candidates for further investigations in order to identify novel G6PD inhibitors.

### 3.2. In vitro anti-cancer potential of mushroom extracts against lung cancer (A549) cells

To assess the relationship of enzymatic inhibitory activities and anticancer potential of the mushroom extracts, MTT assay was performed in 96-well plate at single dose for each extract with final concentration of 200 µg/ml. The effects of ethanolic extracts from mushrooms on cell viability were evaluated against A549 cancer cells for the determination of cytotoxicity.

The results of MTT assay demonstrates that out of 9 extracts screened, *M. procera* extract was found to be active against lung cancer cells which inhibited the cell growth by >75% at a final concentration of 200 µg/ml. The graphical demonstration of MTT results shows that these extracts showed a differential inhibitory potential at a standard final concentration against A549 cells (Fig. 2).

### 3.3. Calculation of IC<sub>50</sub> values of *Macrolepiota procera* extract against G6PD and A549 lung cancer cells

As *M. procera* have demonstrated significant inhibitory potential against G6PD and exhibited cytotoxic activity against A549 lung cancer cells, thus, *M. procera* extract was tested dose dependently against G6PD and A549 cancer cells at variable concentrations (µg/ml) in re-confirmation assay and dose response curves was obtained to calculate the inhibitory concentrations (IC<sub>50</sub>).

The G6PD activity was measured at our validated substrate concentration of G6P with six different concentrations of the extract and absorbance was measured. The obtained results have shown that *M. procera* extract possess IC<sub>50</sub> value of 0.853 µg/ml against G6PD enzymatic activity (Fig. 3A).

A549 cells were treated with various concentrations of *M. procera* extract (0, 0.7, 1.5, 3.125, 6.25, 12.5, 25, 50, 100, 200 and 400 µg/ml) and from resulting dose-response curve, IC<sub>50</sub> value was calculated. *M. procera* extract has potential to inhibit the growth of A549 cells significantly with IC<sub>50</sub> value of 6.18 µg/ml (Fig. 3B).

### 3.4. In silico based screening of chemical constituents from *Macrolepiota procera*

As ethanolic extract of *M. procera* showed significant inhibitory potential against G6PD enzyme, molecular docking was performed in order to investigate the G6PD binding potential of its already reported chemical compounds (paraben, quercetin, syringic acid, ferulic acid, p-coumaric acid, vanillic acid, cinnamic acid, gallic acid, protocatechuic acid, p-hydroxybenzoic acid, malic acid, and oxalic acid). The structure of phytochemicals were retrieved from PubChem software and docked into the two regions of G6PD (NADP<sup>+</sup>-co-enzyme & G6P-substrate binding site). The obtained MolDock Scores are presented in Table 4. These results indicate

**Table 2**  
Preliminary screening of methanolic mushroom extracts for the identification of G6PD and 6PGD inhibitors.

Sr. no.	Mushroom name	Common name	Family	G6PD inhibition	6PGD inhibition
1.	<i>Agaricus arvensis</i>	Horse mushroom	Agaricaceae	+	–
2.	<i>Inocybe geophylla</i>	White fibercap	Inocybaceae	–	+
3.	<i>Lactarius deliciosus</i>	Red pine mushroom	Russulaceae	+	+
4.	<i>Lepista nuda</i>	Wood blewit	Tricholomataceae	–	+
5.	<i>Macrolepiota procera</i>	Parasol mushroom	Agaricaceae	+++	–
6.	<i>Pleurotus eryngii</i>	Oyster mushroom	Pleurotaceae	+	+
7.	<i>Suillus luteus</i>	Slippery jack	Suillaceae	+	+
8.	<i>Terfezia boudieri</i>	Desert truffle	Terfeziaceae	+++	–
9.	<i>Tuber aestivum</i>	Summer truffle	Tuberaceae	–	++

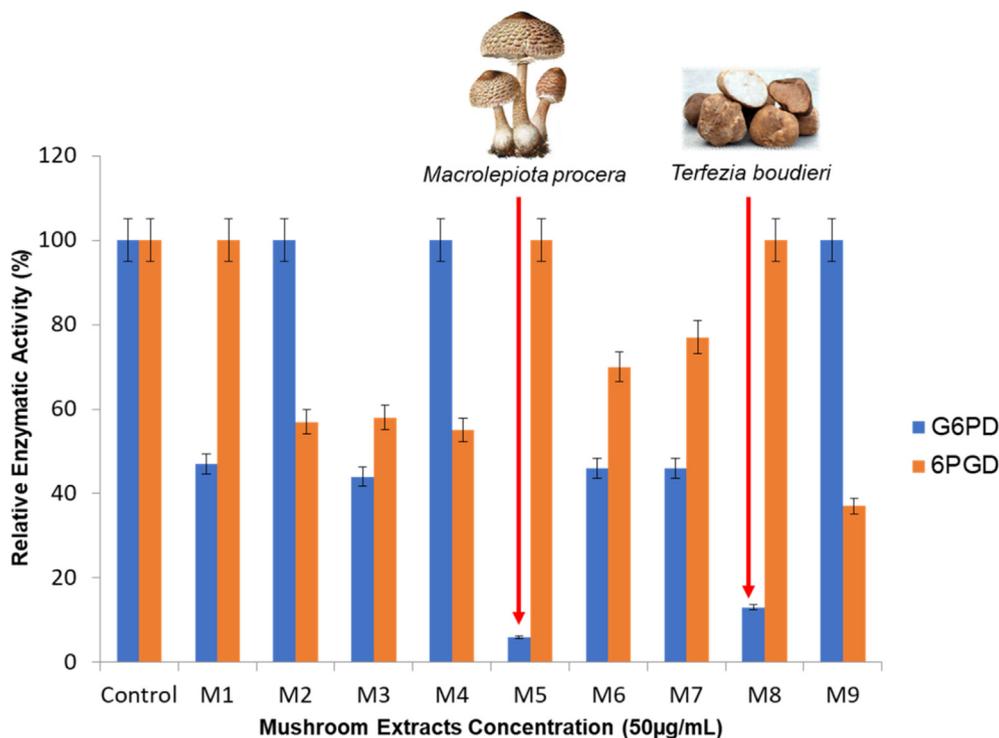
+++ = > 80% inhibition; ++ = 60%–80% inhibition; + = < 60%, inhibition; – = 0% inhibition.

Out of nine extracts screened, three were found to be active on both enzymes, three were selectively active against G6PD while three were selectively active against 6PGD. The percentage of relative enzymatic activities after treatment with various mushroom extracts is presented in Fig. 1.

**Table 3**  
Summary of in vitro enzymatic assay based screening against G6PD and 6PGD.

Total no. of mushrooms extracts screened	Activity against G6PD				Activity against 6PGD			
	Highly active (+++)	Moderately active (++)	Poorly active (+)	Inactive (–)	Highly active (+++)	Moderately active (++)	Poorly active (+)	Inactive (–)
9	2	0	4	3	0	1	5	3

+++ = > 80% inhibition; ++ = 60%–80% inhibition; + = < 60%, inhibition; – = 0% inhibition



**Fig. 1.** Enzymatic activity of ethanolic mushroom extracts against G6PD and 6PGD.

that phytochemicals have a higher binding potential at the binding site of the NADP+ in the enzyme. The overlapping positions of compounds with substrate and coenzyme are demonstrated in Fig. 4.

The interaction modes of paraben, quercetin and syringic acid with the active site of the enzyme are shown in Fig. 5B, Fig. 5C and Fig. 5D respectively. These compounds have potential to bind at the site where the adenosine monophosphate moiety of NADP+ binds. Except for p-coumaric acid and p-hydroxyl benzoic acid, all other phenolic acids have good binding affinities to the same region, as presented in Fig. 5. The docking score for paraben was

found to be highest (-122.027 Moldock Score). This compound was predicted to form four conventional hydrogen bonds with Lys235, Arg487, Arg357 and Lys366 amino acid residues with its carboxylate group, and one hydrogen bond with Asp493 with its –OH groups of benzene ring.

#### 4. Discussion

Fungi belong to an under-explored and understudied group of organisms having enormous potential for the biotechnological

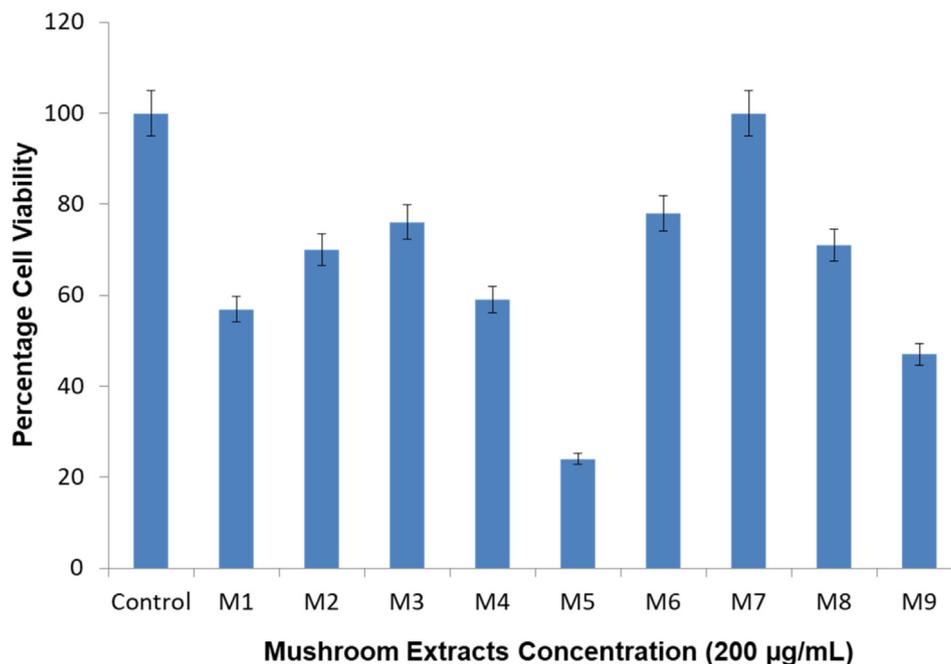


Fig. 2. The percentage of cell viabilities after treatment with various mushroom extracts.

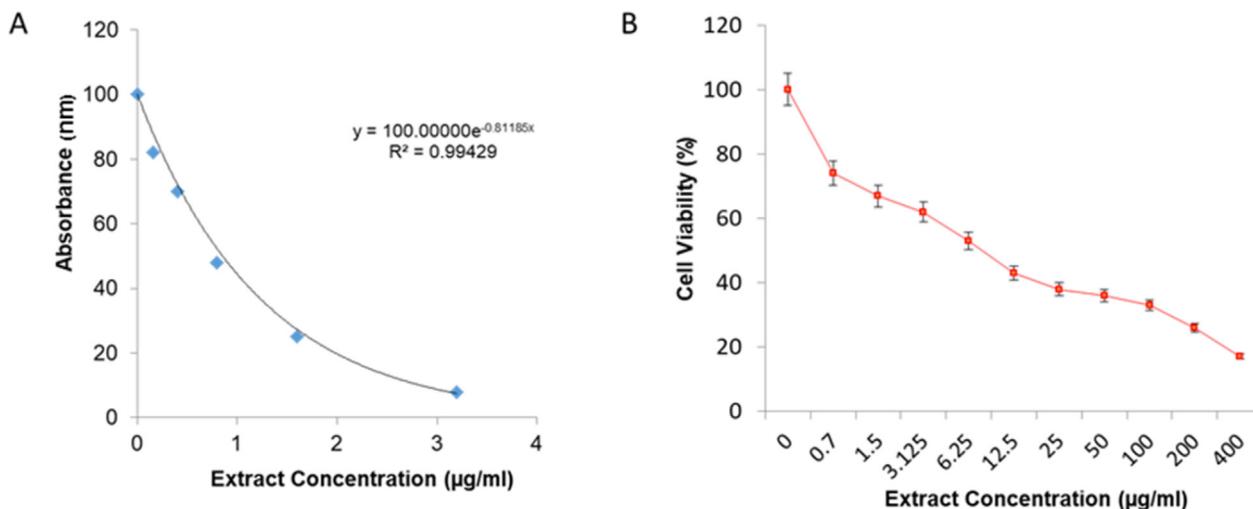


Fig. 3. Dose response curves for calculation of IC<sub>50</sub> values. A) Dose-dependent decrease in activity of G6PD, absorbance after treatment with 0, 1, 2, 3, 4 µg/ml of *M. procera* extract; B) *M. procera* extract caused inhibition of growth in A549 cells. Cancer cells were given treatment of 0, 0.7, 1.5, 3.125, 6.25, 12.5, 25, 50, 100, 200, 400 µg/ml of *M. procera* extract for 24 h.

Table 4  
Binding energies (MolDock Score) of *M. procera*-derived chemical compounds towards G6PD binding sites.

Compound name	PubChem CID	MolDock Score (G6P(substrate) binding cavity) 2BHL	MolDock Score (NADP+ (Co-enzyme) Binding cavity) 2BH9	MolDock Score (NADP+ (Co-enzyme) Binding cavity) 1QKI	References
Paraben	65,492,654	-103.606	-135.566	-122.027	(Erbai et al., 2021)
Quercetin	5,280,343	-85.7291	-111.627	-105.665	(Ozen et al., 2011)
Syringic acid	10,742	-83.1563	-100.271	-80.6785	(Erbai et al., 2021)
Ferulic acid	445,858	-85.9351	-97.9971	-88.7686	(Erbai et al., 2021)
p-coumaric acid	637,542	-77.7975	-91.8341	-79.7101	(Barros et al., 2009)
Vanillic acid	8468	-75.9465	-91.7046	-75.5526	(Erbai et al., 2021)
Cinnamic acid	444,539	-70.7691	-90.6373	-75.8009	(Erbai et al., 2021)
Gallic acid	370	-79.5521	-90.113	-73.9559	(Erbai et al., 2021)
Protocatechuic acid	72	-70.7113	-85.2108	-71.2603	(Erbai et al., 2021)
p-hydroxybenzoic acid	135	-68.7285	-81.8954	-67.2841	(Čirić et al., 2019)
Malic acid	525	-67.6116	-74.9165	-69.3317	(Erbai et al., 2021)
Oxalic acid	971	-47.48	-55.3322	-55.6724	(Chen et al., 2018)

research and industrial products. With the discovery of penicillin, fungi provided the initial recognition of its immense therapeutic potential (Adam et al., 2014). Various fungi-derived anti-cancer lead compounds are currently in preclinical and clinical developmental stages including iriflufen and aphidicolin (Gomes et al., 2015). However, fungi are still untapped source of novel therapeu-

tic agents and it is worthwhile to investigate the potential of fungal species for the discovery of therapeutically active fungal metabolites against various diseases (Padmathilake et al., 2017).

In an attempt to explore the role of fungi for the treatment of cancer, various mushrooms extracts were tested for their potential to reprogram tumor metabolism in this study.

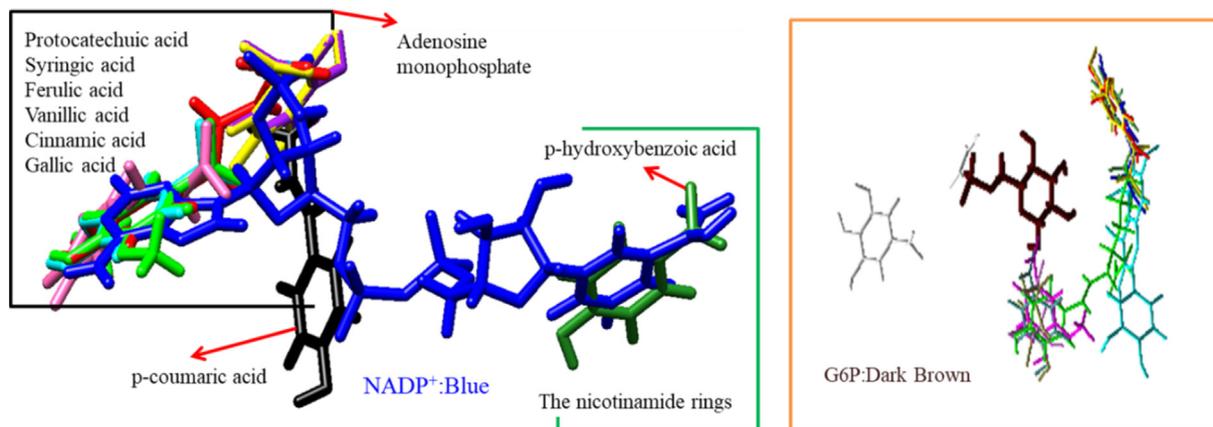


Fig. 4. The overlapping of phenolic acids with natural substrate and coenzyme at active site of G6PD.

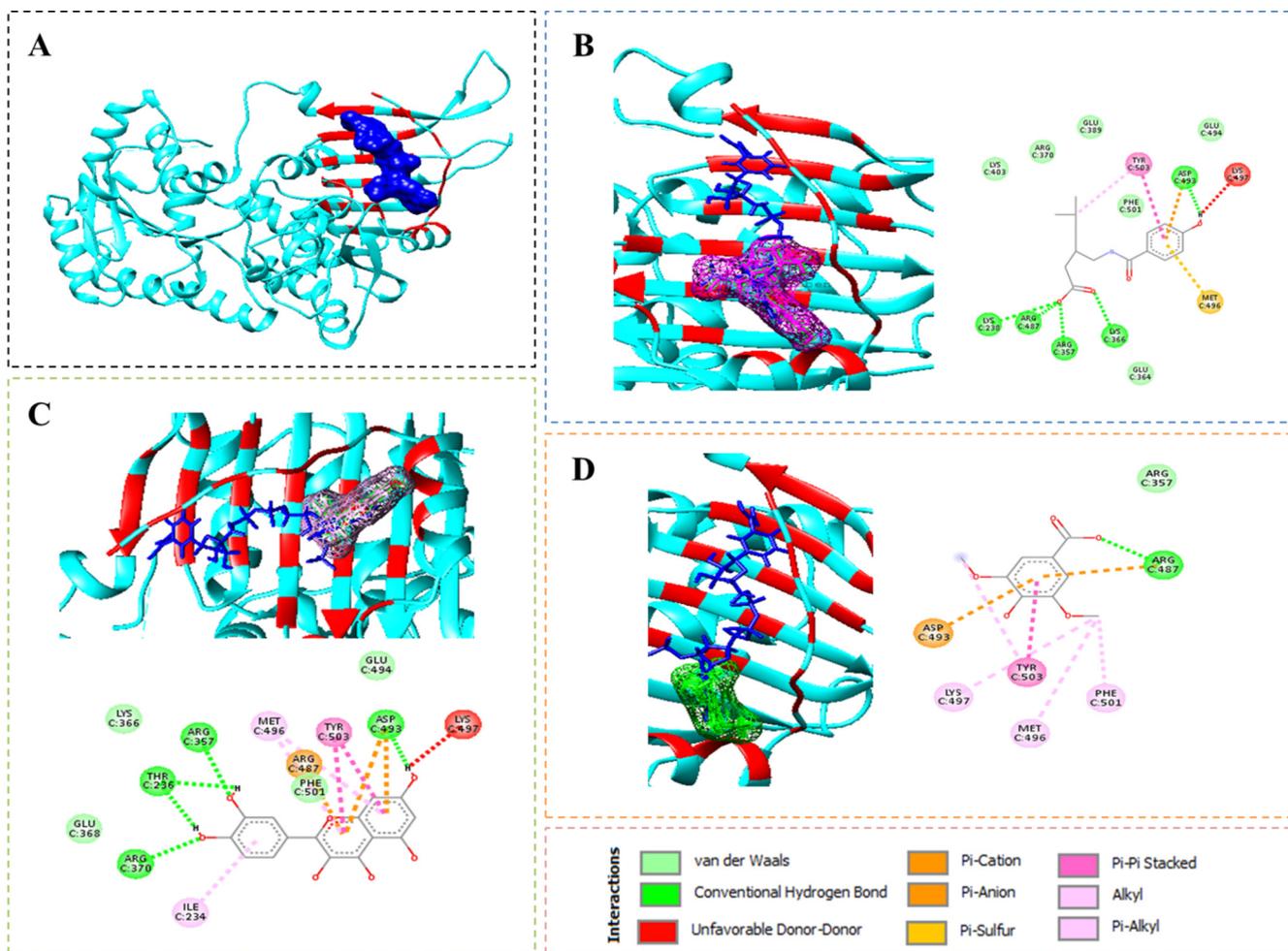


Fig. 5. The comparative binding modes between NAD<sup>+</sup> and three compounds inside the active site of the G6PD, A) References ligand NAD<sup>+</sup> (blue) and amino acids (red) at the G6PD active cavity, B) The binding mode and 2D interaction map of paraben, C) The binding mode and 2D interaction map of quercetin, D) The binding mode and 2D interaction map of syringic acid.

During recent years, targeting metabolic reprogramming of tumor cells has emerged as novel and selective approach for cancer therapy (Fan et al., 2021). Among various reprogrammed metabolic pathways of cancer cells, pentose phosphate pathways (PPP) is especially critical for the rapid proliferation of cancer cells as it generates raw materials for macromolecules biosynthesis as well as redox regulators for cancer cells (Payen et al., 2016). PPP's first-rate limiting enzyme, glucose-6-phosphate dehydrogenase, provides reducing power and ribose phosphate to the cell for redox balance and biosynthesis of nucleotides and lipids. The role of G6PD in cancer cell survival, proliferation, and metastasis has been well established by recent research investigations. G6PD has also been reported to be highly expressed in various cell lines of lung cancer which provide further rationale for targeting G6PD as novel anti lung cancer therapy. Thus, it is of great interest to develop and identify the inhibitors of G6PD from fungi which could serve as ideal therapeutic agents for the treatment of lung cancer.

After screening of edible mushroom extracts, we identified *M. procera* and *T. boudieri* extracts as G6PD activity inhibitors at a final dose of 50 µg/ml. In the subsequent screening of crude extracts for the evaluation of their anti-cancer potential, *M. procera* was identified as the only one demonstrating promising activity against lung cancer cells. Thus, this study identified *M. procera* as a natural product that targets G6PD to exert its anti-lung cancer effects. Previous studies have indicated that a marine fungus *Diaporthe* sp. exhibit G6PD inhibitory activity with anti-cancer potential against HeLa cells. The extract from this potentially active fungus contains bioactive compound, Mycoepoxydiene, which downregulates the G6PD expression (Jin et al., 2017). Another study reported the inhibition of G6PD after the treatment of natural extract obtained from licorice root extract (Kamel et al., 2020). Previous research studies have also reported the anticancer potential of *M. procera* extract against lung and colon cancers (Arora et al., 2013). Our results are also found to be concordant with the previous studies which demonstrate that inhibition of G6PD in cervical (HeLa), lung (A549), breast (MCF-7) and hepatocellular (HepG2) tumor cells halts the proliferation of cancer cells (Fang et al., 2016; Yang et al., 2019). The results of in silico based screening revealed lysine, arginine and aspartate as common interacting amino acids forming hydrogen bonds with NADP<sup>+</sup> binding site of G6PD. It is previously reported that the 2'-phosphate of NADP<sup>+</sup> makes hydrogen bonds with arginine and lysine (Au et al., 2000). Thus, interactions of paraben with these amino acids can make an important contribution to the blocking of enzyme activity of the molecule. The conformational energy of paraben was minimized by the presence of one pi-sulfur interaction with Met496, and one pi-anion interaction with Met49. While Lys403, Arg370, Glu389, Phe501, Glu494, and Glu364 contributed towards strong binding via van der Waals interactions, which further validates the contribution of these amino acid residues in binding towards G6PD.

## 5. Conclusions

With mushroom extracts screening, this study identified *M. procera* extract as selective inhibitor of G6PD. Although *M. procera* extract has been previously reported as anticancer agent, the underlying mechanism was not known. To our knowledge, this is the first study which revealed the anticancer potential of *M. procera* against A549 cells via G6PD inhibition. In addition, the findings from in silico screening suggest that paraben, quercetin and syringic acid should be further investigated for their G6PD inhibitory potential via in vitro assay. Overall, this study endorses the use of this edible mushroom specie as anti-cancer therapeutic agent, however, further *in vivo* studies are recommended to validate the potential of *M. procera* against lung cancer.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

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## References

- Adam, M., Heuer, H., Hallmann, J., Berg, G., 2014. Bacterial antagonists of fungal pathogens also control root-knot nematodes by induced systemic resistance of tomato plants. *PLoS One* 9 (2), e90402. <https://doi.org/10.1371/journal.pone.0090402>. <https://doi.org/10.1371/journal.pone.0090402.g002>. <https://doi.org/10.1371/journal.pone.0090402.g003>. <https://doi.org/10.1371/journal.pone.0090402.t001>. <https://doi.org/10.1371/journal.pone.0090402.t002>. <https://doi.org/10.1371/journal.pone.0090402.t003>. <https://doi.org/10.1371/journal.pone.0090402.t004>.
- Arora, S., Goyal, S., Balani, J., Tandon, S., 2013. Enhanced antiproliferative effects of aqueous extracts of some medicinal mushrooms on colon cancer cells. *Int. J. Med. Mushrooms* 15 (3), 301–314.
- Au, S.W.N., Gover, S., Lam, V.M.S., Adams, M.J., 2000. Human glucose-6-phosphate dehydrogenase: the crystal structure reveals a structural NADP<sup>+</sup> molecule and provides insights into enzyme deficiency. *Structure* 8 (3), 293–303.
- Bade, B.C., Dela Cruz, C.S., 2020. Lung Cancer 2020. *Clin. Chest Med.* 41 (1), 1–24.
- Barros, L., Dueñas, M., Ferreira, I.C.F.R., Baptista, P., Santos-Buelga, C., 2009. Phenolic acids determination by HPLC-DAD-ESI/MS in sixteen different Portuguese wild mushrooms species. *Food Chem. Toxicol.* 47 (6), 1076–1079.
- Braun, S., Hanselmann, C., Gassmann, M.G., auf dem Keller, U., Born-Berclaz, C., Chan, K., Kan, Y.W., Werner, S., 2002. Nr2 transcription factor, a novel target of keratinocyte growth factor action which regulates gene expression and inflammation in the healing skin wound. *Mol. Cell Biol.* 22 (15), 5492–5505.
- Chan, B., VanderLaan, P.A., Sukhatme, V.P., 2013. 6-Phosphogluconate dehydrogenase regulates tumor cell migration in vitro by regulating receptor tyrosine kinase c-Met. *Biochem. Biophys. Res. Commun.* 439 (2), 247–251.
- Chen, H.-P., Zhao, Z.-Z., Li, Z.-H., Huang, Y., Zhang, S.-B., Tang, Y., Yao, J.-N., Chen, L., Isaka, M., Feng, T., Liu, J.-K., 2018. Anti-Proliferative and Anti-Inflammatory Lanostane Triterpenoids from the Polish Edible Mushroom *Macrolepiota procera*. *J. Agric. Food Chem.* 66 (12), 3146–3154.
- Cho, E.S., Cha, Y.H., Kim, H.S., Kim, N.H., Yook, J.I., 2018. The Pentose Phosphate Pathway as a Potential Target for Cancer Therapy. *Biomol. Ther. (Seoul)* 26 (1), 29–38.
- Čirić, A., Kruljević, I., Stojković, D., Fernandes, A., Barros, L., Calheta, R.C., Ferreira, I.C.F.R., Soković, M., Glamočlija, J., 2019. Comparative investigation on edible mushrooms *Macrolepiota mastoidea*, *M. rhacodes* and *M. procera*: functional foods with diverse biological activities. *Food Funct.* 10 (12), 7678–7686.
- Dang, C.V., 2018. Cancer Metabolism: the Known, Unknowns. *Biochim. Biophys. Acta Rev. Cancer* 1870 (1), 1. <https://doi.org/10.1016/j.bbcan.2018.07.006>.
- Enzo, E. et al., 2015. Aerobic glycolysis tunes YAP/TAZ transcriptional activity. *EMBO J.* 34 (10), 1349–1370.
- Erbia, E.H., da Silva, L.P., Saidi, R., Lamrani, Z., Esteves da Silva, J.C.G., Maouni, A., 2021. Chemical Composition, Bioactive Compounds, and Antioxidant Activity of Two Wild Edible Mushrooms *Armillaria mellea* and *Macrolepiota procera* from Two Countries (Morocco and Portugal). *Biomolecules* 11 (4), 575. <https://doi.org/10.3390/biom11040575>.
- Fan, C., Zhang, S., Gong, Z., Li, X., Xiang, B.O., Deng, H., Zhou, M., Li, G., Li, Y., Xiong, W., Zeng, Z., Li, X., 2021. Emerging role of metabolic reprogramming in tumor immune evasion and immunotherapy. *Sci. China Life Sci* 64 (4), 534–547.
- Fang, Z., Jiang, C., Feng, Y.i., Chen, R., Lin, X., Zhang, Z., Han, L., Chen, X., Li, H., Guo, Y., Jiang, W., 2016. Effects of G6PD activity inhibition on the viability, ROS generation and mechanical properties of cervical cancer cells. *Biochim. Biophys. Acta* 1863 (9), 2245–2254.
- Gomes, N.G. et al., 2015. Can Some Marine-Derived Fungal Metabolites Become Actual Anticancer Agents? *Mar. Drugs* 13 (6), 3950–3991.
- Hetland, G., Tangen, J.-M., Mahmood, F., Mirlashari, M.R., Nissen-Meyer, L.S.H., Nentwich, I., Therikelsen, S.P., Tjønnfjord, G.E., Johnson, E., 2020. Anti-Inflammatory and Antiallergic Effects of Agaricus blazei Mushroom Extract and the Related Medicinal Basidiomycetes Mushrooms, *Hericium erinaceus* and *Grifola frondosa*: A Review of Preclinical and Clinical Studies. *Nutrients* 12 (5), 1339. <https://doi.org/10.3390/nu12051339>.
- Jeitler, M. et al., 2020. Significance of Medicinal Mushrooms in Integrative Oncology: A Narrative Review. *Front. Pharmacol.* 11, 580656.
- Jin, K., Li, L., Sun, X., Xu, Q., Song, S., Shen, Y., Deng, X., 2017. Mycoepoxydiene suppresses HeLa cell growth by inhibiting glycolysis and the pentose phosphate pathway. *Appl. Microbiol. Biotechnol.* 101 (10), 4201–4213.
- Kamel, N.M., Abdel-Motaal, F.F., El-Zayat, S.A., 2020. Endophytic fungi from the medicinal herb *Euphorbia geniculata* as a potential source for bioactive metabolites. *Arch. Microbiol.* 202 (2), 247–255.

- Kotaka, M., Gover, S., Vandeputte-Rutten, L., Au, S.W.N., Lam, V.M.S., Adams, M.J., 2005. Structural studies of glucose-6-phosphate and NADP<sup>+</sup> binding to human glucose-6-phosphate dehydrogenase. *Acta Crystallogr. Sect. D Biol. Crystallogr.* 61 (5), 495–504.
- Majeed, F.A., Azeem, A.R., Farhan, N., 2019. Lung cancer in Pakistan, where do we stand? *J. Pak. Med. Assoc.* 69 (3), 405–408.
- Molegro, A., 2019. MVD 7.0 Molegro Virtual Docker. DK-8000 Aarhus C, Denmark.
- Nakamura, M., Verboon, J.M., Prentiss, C.L., Parkhurst, S.M., 2020. The kinesin-like protein Pavarotti functions noncanonically to regulate actin dynamics. *J. Cell Biol.* 219 (9). <https://doi.org/10.1083/jcb.201912117>.
- Ozen, T. et al., 2011. Screening of antioxidant, antimicrobial activities and chemical contents of edible mushrooms widely grown in the black sea region of Turkey. *Comb Chem High Throughput Screen* 14 (2), 72–84.
- Padmathilake, K.G.E., Bandara, H.M.S.K.H., Qader, M.M., Kumar, N.S., Jayasinghe, L., Masubuti, H., Fujimoto, Y., 2017. Talarofuranone, a New Talaroconvolutin Analog from the Endophytic Fungus *Talaromyces purpurogenus* from *Pouteria campechiana* Seeds 1934578X1701200 Nat. Prod. Commun. 12 (4). <https://doi.org/10.1177/1934578X1701200406>.
- Payen, V.L., Porporato, P.E., Baselet, B., Sonveaux, P., 2016. Metabolic changes associated with tumor metastasis, part 1: tumor pH, glycolysis and the pentose phosphate pathway. *CellMol. Life Sci.* 73 (7), 1333–1348.
- Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., Ferrin, T.E., 2004. UCSF Chimera—a visualization system for exploratory research and analysis. *J. Comput. Chem.* 25 (13), 1605–1612.
- Preuss, J., Maloney, P., Peddibhotla, S., Hedrick, M.P., Hershberger, P., Gosalia, P., Milewski, M., Li, Y.L., Sugarman, E., Hood, B., Suyama, E., Nguyen, K., Vasile, S., Sergienko, E., Mangravita-Novo, A., Vicchiarelli, M., McAnally, D., Smith, L.H., Roth, G.P., Diwan, J., Chung, T.D.Y., Jortzik, E., Rahlfs, S., Becker, K., Pinkerton, A. B., Bode, L., 2012. Discovery of a *Plasmodium falciparum* glucose-6-phosphate dehydrogenase 6-phosphogluconolactonase inhibitor (R, Z)-N-((1-ethylpyrrolidin-2-yl)methyl)-2-(2-fluorobenzylidene)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazine-6-carboxamide (ML276) that reduces parasite growth in vitro. *J. Med. Chem.* 55 (16), 7262–7272.
- Rasul, A. et al., 2012. Cytotoxic effect of evodiamine in SGC-7901 human gastric adenocarcinoma cells via simultaneous induction of apoptosis and autophagy. *Oncol Rep* 27 (5), 1481–1487.
- Vazquez, A., Kamphorst, J.J., Markert, E.K., Schug, Z.T., Tardito, S., Gottlieb, E., 2016. Cancer metabolism at a glance. *J. Cell Sci.* 129 (18), 3367–3373.
- Yang, H.C. et al., 2019. The Redox Role of G6PD in Cell Growth, Cell Death, and Cancer. *Cells* 8 (9).
- Yang, X., Peng, X., Huang, J., 2018. Inhibiting 6-phosphogluconate dehydrogenase selectively targets breast cancer through AMPK activation. *Clin. Transl. Oncol.* 20 (9), 1145–1152.
- Zhang, H.S. et al., 2019. Nrf2 promotes breast cancer cell migration via up-regulation of G6PD/HIF-1 $\alpha$ /Notch1 axis. *J. Cell Mol. Med.* 23 (5), 3451–3463.
- Zmijewski, M.A., 2019. Vitamin D and Human Health. *Int. J. Mol. Sci.* 20 (1), 145. <https://doi.org/10.3390/ijms20010145>.