

Original Research Article

Anti-aging activity of *Xylaria striata* in *Drosophila melanogaster*

ABSTRACT

Aims: To evaluate the application potential of *Xylaria striata* in anti-aging field.

Methodology: Extracting the fruit body of *Xylaria striata* by ultrasonic-assisted extraction method. In this study, *Drosophila melanogaster* was used as an anti-aging organism material. After feeding with different concentrations of extract, the natural survival time, survival time under oxidative stress and survival time under UV irradiation of *Drosophila melanogaster* were all measured. In addition, the *in vivo* activity of SOD, CAT and MAD, protein concentration and body weight were determined to evaluate the anti-aging effect of ethanol extract from *Xylaria striata*.

Results: The results showed that the ethanol extract of *Xylaria striata* could extend the lifespan of *Drosophila melanogaster* under both irradiation and oxidative stress condition. And the ethanol extract could enhance the activity of CAT in *Drosophila melanogaster*, especially at concentration of 50 μ M, and the content of MAD in *Drosophila melanogaster* were decreased significantly.

Conclusion: This study clarified the anti-aging activity of *Xylaria striata* in *Drosophila melanogaster* and it would provide some theoretical basis for its further development and utilization in anti-aging drugs and health food.

Keywords: *Xylaria striata*; anti-aging; *Drosophila melanogaster*; lifespan.

1 INTRODUCTION

The higher fungi contain compounds with many novel structure, which have variety and remarkable biological activity^[1]. Because of potential medicinal and economic value, they have drawn an increasing attention and become a highlight research field of science and industry in recent years.

The genus *Xylaria*, a big family of mycomycetes, has great value in application according to a large amount of literature reports. There were many kinds of compounds such as terpenoids, sterols, alkaloids, polyketones, polysaccharides, cyclic peptides and carboxylic acids^[2-4] had been isolated from the *Xylaria* genus, which exhibited antioxidant, antimicrobial, antitumor, enzyme inhibitory abilities. *Xylaria striata* Pat. 1887, mainly grows on decayed barks and lived roots of broad-leaved woodland. It was used as folk medicine in China^[5]. After finding its diverse biological activities in the preliminary screening of large numbers of edible and medicinal mushrooms, our research group started a systematic study on *Xylaria striata* from cultivation method to chemical components since 2013^[6-7]. These research results clarified its effect of anti-animal and plant pathogens^[8], anti-tumor^[9], and promoting sleep^[10].

Furthermore, another mushroom from the same genus named *Xylaria nigripes* has been widely used to prevent and treat senile diseases in China. It can promote the effect of

antidepressants^[11] and alleviate depressive symptoms in patients with epilepsy^[12]. Hence, the main objectives of this study were to evaluate the anti-aging activity of *Xylaria striata* using *Drosophila melanogaster* as the model organism by assessing the lifespan of flies, the activity of SOD and CAT, the content of MDA and protein and its stress resistant ability under H₂O₂ and UV irradiation treatment.

2 MATERIALS

2.1 Biomaterial

Xylaria striata was obtained in 2013 from Qingyi town, Mianyang, Sichuan province of China, and identified by professor Xin-sheng He of microbiology, Southwest University of Science and Technology. The voucher specimen was preserved in the Microbiology Laboratory of the same university. *Drosophila melanogaster*, reared under the condition of temperature 25 ± 1 °C, humidity 60%-80%.

2.2 Reagents

Protein content determination kit, superoxide dismutase (SOD) determination kit, catalase (CAT) determination kit, and malondialdehyde (MDA) content determination kit are all purchased from Nanjing Jiancheng bioengineering institute; Corn flour, agar and yeast extract was purchased from Beijing Aobaoing Bio-Tech Co., Ltd.; Ethyl ether, ethanol, 30% hydrogen peroxide and other reagents were purchased from ChengDu Chon Chemicals Co., Ltd.; All reagents are analytical pure grade and pure water is made in the laboratory.

2.3 Equipments

Biochemical incubator (SPX- 80B, Shanghai Kuntian instrument Co., Ltd.), Thermo Scientific Microplate Reader (Multiskan Spectrum, Thermo Fisher), Ultraviolet lamp (LP-GDZJ40W, LONGPRO CO., Ltd.), Ultrasonic cleaner (KQ-200KDE, Kunshan Ultrasonic Instrument Co., Ltd.).

3 METHODS

3.1 Sample pretreatment

The cultivated fruiting body^[13] of *Xylaria striata* was dried at 50 °C and ethanol-assisted ultrasonic extraction process was carried out at the ratio of material to liquid (1:30 g/mL) for three times, 10 min of each. Then, the extract was obtained by vacuum distilling of the filtered supernatant at 65 °C and preserved at 4 °C until use.

3.2 Diet preparation and Fly husbandry

The wild-type *Drosophila melanogaster* Canton-S (CS) flies, selected as the experiment organism for later assay, were reared at temperature 25 ± 1 °C, humidity 60%-80%. Before the experiment, *Drosophila melanogaster*s were mass reared in 500 mL erlenmeyer flask containing 100 mL of standard cornmeal diet^[14] (13% cornmeal w/v, 8% sugar w/v, 2.4% Agar w/v, 1.6% yeast extract w/v and 0.4% acetic acid v/v). To avoid overcrowding, 200 flies of each bottle was enough. When the progeny drosophila was going to hatch, all adult flies would be transferred to the new culture bottle. Afterwards, the flies eclosed within 24 h were collected and segregated according to their sex.

3.3 Longevity assay

Refers to Proshkina *et al*^[15], 10 newly eclosed male flies, reared on a standard cornmeal medium with ethanol extract from *Xylaria striata*. The extract was dissolved in water and mixed with the standard diet at final concentrations of 50, 100 and 200 µM. Negative control diets contained only water while positive control contained ascorbic acid at final

concentrations of 100 μM . During the rearing process, diet was replaced with fresh medium every five days and the numbers of alive flies were recorded every two days. The test was stopped until all flies were dead. All the treatments were carried out with 3 replicate. Median lifespan was calculated using the method reported by Kaplan–Meier^[16] previously.

3.4 Oxidative stress resistance

The situation of group dividing is the same as Longevity assay. Newly eclosed male flies were fed on standard diet with the extract from *Xylaria striata* for 25 days, followed by an oxidative challenge of 9% **H2O2** in 6% glucose solution on filter paper strips^[17]. The number of death was recorded every 4 h until all flies were dead. Median lifespan was calculated as the same as 3.3.

3.5 Irradiation resistance

The flies were treated at a constant distance of 10 cm from the ultraviolet lamp and irradiation time was 20 min^[18]. We strictly controlled the surrounding temperature at 25 $^{\circ}\text{C}$ to decrease heat stroke from the lamp during irradiation. Then, the flies were transferred to vials. Dead flies were counted every day until all flies died. Life span was studied in the same way.

3.6 SOD、CAT、MDA、Protein content and weight assays

After 25 days of feeding, flies were transferred into empty tube for 2 hours. After anesthesia, the flies were homogenized in an ice bath at the ratio of weight to normal saline at 1:19 (g:mL). The supernatant was obtained after the homogenate was centrifuged in 2500 $\text{r} \cdot \text{min}^{-1}$ for 20 min at 4 $^{\circ}\text{C}$. The activity of CAT(catalase) and SOD(superoxide dismutase), the content of MDA(malondialdehyde) and protein were determined by the Thermo Scientific Microplate Reader according to the instruction described by the kit. Flies were anesthetized and weighed every ten flies. For each concentration, 3 replicate were set up.

3.7 Statistical Analysis

All the data collected were repeated 3 times, and the data were displayed in the form of means \pm SD. The statistical analysis was performed with SPSS 20.0 software, and the difference analysis between groups was performed by One-way ANOVA analysis. Significant differences were expressed by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

4 RESULTS AND DISCUSSION

4.1 Longevity assay

Drosophila melanogaster has been widely used in anti-aging experiments as a model organism because of its short lifespan and easy reproduction^[19]. The anti-aging ability of the drugs or its toxicity can be reflected at a certain level by comparing the lifespan of *Drosophila melanogaster* before and after feeding the drugs. As shown in Table 1, the ethanol extract of *Xylaria striata* can significantly shorten the mean lifespan of *Drosophila melanogaster*, and with the increasing of the dosage, the more significant the shorten effect is. In addition, the maximum lifespan and half survival time of *Drosophila melanogaster* were also reduced. Therefore, there may be some biologically toxic substances in the ethanol extracts of *Xylaria striata*. It has been reported by Yuan *et al.*^[8] that *Xylaria striata* has an inhibitory effect on plant and animal pathogens, which can also prove that it may contain some toxic substances.

Table 1 The effect of *Xylaria striata* on lifespan

Treatment	Concentration (μM)	Max \pm SD (day)	H \pm SD (day)	M \pm SD (day)
Control	-	45.3 \pm 1.2	35.7 \pm 1.6	30.6 \pm 2.1

	50	32±0.9*	20±2**	28.2±0.15
<i>Xylaria striata</i>	100	41.3±6.2	31.3±4.7	34.1±3.7
	200	38±4.3	15.3±1.9***	24.2±2.1**
Ascorbic acid	100	48.9±3.2	39.0±3.4**	37.7±0.7*

Note: Max, maximum lifespan, days; M, mean lifespan, days; H, half survival lifespan; *p < 0.05, **p < 0.01, ***p<0.001

4.2 Oxidative stress resistance

In vivo, hydrogen peroxide reacts with oxygen to produce hydroxyl radicals, which can induce acute oxidative stress damage and shorten the survival time of *Drosophila melanogaster*^[20]. As shown in Table 2, the ethanol extract of *Xylaria striata* could prolong the maximum lifespan, half survival time and mean lifespan of *Drosophila melanogaster* under oxidative stress. When the concentration of extract was 100 µM, the maximum lifespan and the mean lifespan were obviously extended, and the prolongation of the mean lifespan was extremely significant at the concentration of 200 µM.

Table 2 The effect of oxidative stress on lifespan

Treatment	Concentration (µM)	Max±SD (h)	H±SD (h)	M±SD (h)
Control	0	23.3±3.1	16.7±1.1	17.2±0.6
	50	22±2	17±1.3	17±0.6
<i>Xylaria striata</i>	100	29.3±3.3*	18±4	18.3±0.3*
	200	21.3±2.3	15.3±1.1	18.7±0.3**
Ascorbic acid	100	26±2	18.7±2.3	18.5±0.5**

Note: *p < 0.05, **p < 0.01, ***p<0.001

4.3 UV irradiation resistance

Ultraviolet radiation can cause changes in some physiological functions and damages the body. Herein, we determined if the *Xylaria striata* could repair UV irradiation damage by observing the lifespan changes of *Drosophila melanogaster* under UV irradiation stress. According to a previous report, the sensitivity of *Drosophila melanogaster* to ultraviolet light was closely related to the melanin content^[21]. So, this method could also show the effect of the tested agent on melanin production in *Drosophila melanogaster*. As can be shown in Table 3, a low dose of extract could significantly prolong the lifespan of *Drosophila melanogaster* after UV irradiation. Medium, high dose groups could also prolong lifespan, but the effect was inferior to the low dose group. This may be due to the variety of substances in the extract. As the increasing of dose, the increasing biotoxicity resulted in a decrease in lifespan. In addition, we found ascorbic acid can't prolong the lifespan, which indicated that it unable repaired the radiation damage.

Table 3 Effect of UV irradiation on lifespan

Treatment	Concentration (µM)	Max±SD (day)	H±SD (day)	M±SD (day)
Control	0	12.5±0.7	6±1.3	5.2±0.4
	50	17±1.4*	11.5±0.7*	10.4±2.3*
<i>Xylaria striata</i>	100	15±1.4	10±2.8	8.5±1.5
	200	14.5±1.5	9.3±2.9	9±2.5
Ascorbic acid	100	12.5±0.7	9.5±0.7	9.4±0.4

Note: *p < 0.05, **p < 0.01, ***p<0.001

4.4 SOD、CAT、MAD, protein content and weight assay

After feeding on the extract, the overall living condition can be indirectly reflected by determining the weight and protein content of *Drosophila melanogaster*. In Table 4, there were no significant changes in the weight and protein content of flies.

Superoxide dismutase (SOD), one kind of free radical enzyme, can scavenge the superoxide anion in body, and prevent the body from being oxidized. Hydrogen peroxide reacts with oxygen can produce hydroxyl radicals, which are harmful to the body. However, hydrogen peroxide decomposition *in vivo* can be catalyzed by catalase (CAT) to reduce the generation of hydroxyl free radicals^[22]. Hence, SOD and CAT are two kinds of representative enzymes in antioxidant system. The activities of these two enzymes can indirectly evaluate the free radical scavenging ability of the organism^[23].

Malondialdehyde (MDA) is a kind of lipid peroxidation product produced by the reaction of free radicals and unsaturated fatty acids. MDA residues in body can be further cross-linked with proteins and peptides to accelerate the aging of the body^[24].

As can be seen in Table 4, The ethanol extract of *Xylaria striata* has no positive effect on SOD. But the extract at low dose can promote the activity of CAT to reduce the free radicals. Furthermore, the extract can also significantly decrease the content of MDA which is consistent with the content of free radicals. It is implied that the MDA content can also indirectly reflect the content of free radicals and the degree of lipid peroxidation. So, it is conducted that the ethanol extract of *Xylaria striata* keeps body much away from peroxidation and prevent it from aging too quickly.

Table 4 Effect of *Xylaria striata* on SOD/CAT/MDA

Treatment	Concentration (μM)	Protein content (g/L)	CAT (U/mL)	MDA (nmol/mL)	SOD (U/mg)	Weight (mg/10 flies)
Control	0	1.08±0.16	61.91±5.92	2.65±0.40	43.21±8.5	6.7±0.76
<i>Xylaria striata</i>	50	1.32±0.23	87.47±11.34*	0.9±0.27***	34.59±6.71	6.9±0.32
	100	1.37±0.22	69.46±6.94	1.75±0.11**	36.51±6.91	6.6±0.83
	200	1.3±0.23	58.49±10.34	1.03±0.26***	29.45±2.91	6.2±0.26
Ascorbic acid	100	1.37±0.23	92.27±13.23*	2.65±0.27	34.11±6.2	5.9±0.07

Note: *p < 0.05, **p < 0.01, ***p<0.001

5 CONCLUSIONS

From the previous report that can promote pentobarbital-induced sleep by not only increasing the number of falling asleep and prolonging sleeping time but also reducing sleep latency^[25], this mushroom had potential to be as a functional food used in the field of geriatrics. Above all, the anti-aging activity of *Xylaria striata* was evaluated by measuring the survival time under various conditions, the activity of SOD/CAT and the content of MAD/protein of *Drosophila melanogaster*. The results showed that, although the ethanol extract of *Xylaria striata* could shorten the lifespan of *Drosophila melanogaster* under natural conditions which indicated its biologically toxic, it could extend the lifespan of flies in the longevity test of two stress models by repair both ultraviolet radiation damage and hydrogen peroxide oxidative stress damage. The results of enzyme activity and MAD content showed that the ethanol extract of *Xylaria striata* could not only block the source of free radicals but also eliminate the reaction products of free radicals.

213 **COMPETING INTERESTS**

214 Authors have declared that no competing interests exist.

215

216 **REFERENCES**

- 217 1. Zhao JD. Chinese fungi(The third volume). 1th ed. Beijing; Science Press, 1998.
- 218 2. Gao C, Luo J, Liu X, Ma L, Yuan XH. Recent advances in the studies of chemical
219 compositions and bioactivities of the genus *Xylaria*. *Mycosystema*. 2016,35(07):767-781.
220 DOI:10.13346/j.mycosystema.150061
- 221 3. Macias-Rubalcava M, Sanchez-Fernandez RE. Secondary metabolites of endophytic
222 *Xylaria* species with potential applications in medicine and agriculture. *World Journal Of*
223 *Microbiology & Biotechnology*. 2017, 33(01):15. DOI: 10.1007/s11274-016-2174-5
- 224 4. Song F, Wu SH, Zhai YZ, Xuan QC, Wang T. Secondary Metabolites from the Genus
225 *Xylaria* and Their Bioactivities. *CHEMISTRY & BIODIVERSITY*. 2014, 11(05): 673-694.
226 DOI: 10.1002/cbdv.201200286
- 227 5. He XS. Images of mushroom fungus in SiChuan. 1th ed. Bei Jing: Science Press; 2011.
- 228 6. Liu X, Gao C, Zhong YT, Yuan XH, He XS, Ma L. Optimization of submerged culture
229 conditions for *Xylaria striata* mycelium. *Journal of Chinese medicinal materials*. 2014,
230 37(08):1317-21.
- 231 7. Lei CW, Yang ZQ, Zeng YP, Zhou Y, Huang Y, He XS, et al. Xylastriasan A, a new
232 cytochalasan from the fungus *Xylaria striata*. *Natural product research*. 2018, 32(01):7-
233 13. DOI: 10.1080/14786419.2017.1324959
- 234 8. Zhang CJ, Liu X, Zhu P, Liu XL, He XS. Antimicrobial Activities of Ethanol
235 Extracts from Six Species of Higher Fungi against Plant Pathogen. *Journal of*
236 *Anhui Agricultural Sciences*. 2013, 41(12):5289-5294. DOI:
237 10.13989/j.cnki.0517-6611.2013.12.108
- 238 9. Yuan XH, Zhang CJ, Zheng RL, Xu JR, Liu Z, He XS. Screening of Anti-tumor
239 Activities of Ethanol Extracts from Six Species of Higher Fungi. *Journal of*
240 *Southwest University of Science and Technology*, 2013, 28(3):95-97.
- 241 10. Lie CW. Studies on the chemical constituents of *Xylaria striata* and their
242 bioactivities. *Southwest University of Science and Technology*, 2017.
- 243 11. Zheng W, Zhang YF, Zhong HQ, Mai SM, Yang XH, Xiang YT. Wuling Capsule
244 for Major Depressive Disorder: A Meta-analysis of Randomised Controlled Trials.
245 *East Asian archives of psychiatry : official journal of the Hong Kong College of*
246 *Psychiatrists*. 2016, 26(3): 87-97.
- 247 12. Peng WF, Wang X, Hong Z, Zhu GX, Li BM, Li Z, et al. The anti-depression
248 effect of *Xylaria nigripes* in patients with epilepsy: A multicenter randomized
249 double-blind study. *Seizure-european Journal of Epilepsy*. 2015, 29: 26-33. DOI:
250 10.1016/j.seizure.2015.03.014
- 251 13. Hou CL, Sun XL, Tan J, Zhang CX, Yang DS, He XS. Study on Culture of *Xylaria*
252 *striata* Fruit Body. *Food and Fermentation Sciences & Technology*. 2013,49(02):
253 44-46+102. DOI:1674-506X (2013) 02-0044-0003
- 254 14. Chattopadhyay D, Chitnis A, Talekar A, Mulay P, Makkar M, James J, et al.
255 Hormetic efficacy of rutin to promote longevity in *Drosophila melanogaster*.
256 *Biogerontology*. 2017,18(3): 397-411. DOI: 10.1007/s10522-017-9700-1

- 257 15. Proshkina E, Lashmanova E, Dobrovolskaya E, Zemskaya N, Kudryavtseva A,
258 Shaposhnikov M, et al. Geroprotective and Radioprotective Activity of Quercetin,
259 (-)-Epicatechin, and Ibuprofen in *Drosophila melanogaster*. *Frontiers in*
260 *Pharmacology*. 2016, 7: 505. DOI: 10.3389/fphar.2016.00505
- 261 16. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations.
262 *Journal of the American Statistical Association*. 1958, 53(282): 457-481.
- 263 17. Bayliak MM, Lylyk MP, Gospodaryov DV, Kotsyubynsky VO, Butenko NV, Storey
264 KB, et al. Protective effects of alpha-ketoglutarate against aluminum toxicity in
265 *Drosophila melanogaster*. *Comparative biochemistry and physiology. Toxicology*
266 *& pharmacology : CBP*. 2019, 217: 40-53. DOI:10.1016/j.cbpc.2018.11.020
- 267 18. Rajpurohit S, Schmidt PS. Latitudinal Pigmentation Variation Contradicts
268 Ultraviolet Radiation Exposure: A Case Study in Tropical Indian *Drosophila*
269 *melanogaster*. *Frontiers in Physiology*. 2019, 10: 84. DOI:
270 10.3389/fphys.2019.00084
- 271 19. Piper MDW, Partridge L. *Drosophila* as a model for ageing. *BIOCHIMICA ET*
272 *BIOPHYSICA ACTA-MOLECULAR BASIS OF DISEASE*. 2018, 1864(09): 2707-
273 2717. DOI: 10.1016/j.bbdis.2017.09.016
- 274 20. Niki E. Lipid peroxidation products as oxidative stress biomarkers. *Biofactors*.
275 2008, 34: 171-180.
- 276 21. Li HJ, Zhang SF, Li JX, Zhao Z. Anti-UV Irradiation Effect of Melanin Derived
277 from Apricot Kernel Skin in *Drosophila*. *Food Science*. 2012,33(21): 285-289.
- 278 22. Jiao SP, Chen B, Du PG. Anti-lipid peroxidation effect of *Rosa davuriuca*
279 *Pall.* fruit. *Journal of Chinese Integrative Medicine*. 2004, 2(5):364-365.
- 280 23. Zhao J, Chen C, Ma N, Sun ZO, Xue WC, Wang H. The Anti-Aging Effect of
281 Phloridein in Male *Drosophila melanogaster*. *Acta Nutrimenta Sinica*. 2015,
282 37(04): 372-375+383. DOI:10.13325/j.cnki.acta.nutr.sin.2015.04.019
- 283 24. Zhou Q, Zhu L, Zhang D, Li N, Li Q, Dai P, et al. Oxidative Stress-Related
284 Biomarkers in Postmenopausal Osteoporosis: A Systematic Review and Meta-
285 Analyses. *Disease Markers*. 2016, 2016:7067984. DOI: 10.1155/2016/7067984
- 286 25. Lei CW, Yang ZQ, Zeng YP, Zhou Y, Huang Y, He XS, et al. Xylastriasan A, a
287 new cytochalasan from the fungus *Xylaria striata*. *Natural product research*.
288 2018, 32(1): 7-13. DOI: 10.1080/14786419.2017.1324959