ФАРМАКОЛОГИЯ И ФАРМАЦИЯ
PHARMACOLOGY AND PHARMACY

MITIGATION OF INTESTINAL AUTOFLUORESCENCE ACCUMULATION IN *CAENORHABDITIS ELEGANS* TREATED WITH PLANT-BASED NATURAL PRODUCTS

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ABSTRACT

Introduction. *Aging is a complex process related with the gradual diminution in cellular and physiological functions. The geroprotective effect of 10 biologically active substances (BASs) – rutin, squalene, kaempferol, biochanin A, ursolic acid, chlorogenic acid, baicalin, mangiferin, quercetin and trans-cinnamic acid and 5 crude extracts (Ginkgo biloba L., Pulmonaria officinalis L., Scutellaria baicalensis Georgi, Hedysarum neglectum Ledeb. and Panax ginseng C.A. Mey) isolated from medicinal plants of Altai Region of Russia were evaluated for their influence on the accumulation of intestinal autofluorescence material (IAM) using Caenorhabditis elegans model.*
The aim of the study. *IAM facilitates age-related decline and is a non-intrusive biomarker of senescence. This study assessed the impact of different bioactive substances in reducing the build-up of IAM using C. elegans model.*
Materials and methods. *Gravid nematodes were synchronized, and then seeded in 96-well plates to develop to L4-stage. Each BAS in 200 μM, 100 μM, 50 μM and 10 μM concentrations and extracts with a tenth, hundredth and thousandth times-dilution were administered to each well in 6 replicates for each treatment group. On incubation days 1, 5, and 15, adult L4 nematodes underwent spectrofluorometric analysis to determine the effect of the BASs and extracts on IAM accumulation.*
Results. *It was found that quercetin, kaempferol, baicalin, mangiferin, G. biloba and P. officinalis extracts exhibited the most profound inhibition of IAM accumulation compared to the control. It was noteworthy that the 10 μM concentration of mangiferin significantly inhibited IAM accumulation in a manner comparable to the 200 μM of baicalin and 100 μM of quercetin. In addition, the crude extracts of G. biloba and P. officinalis respectively exhibited 2.8- and 1.8-fold decrease in IAM accumulation.*
Discussion. *The accretion of IAM is inversely proportional to longevity. Thus, the BASs identified in this study to modulate IAM accumulation could serve as important precursors or active ingredients for the pharmacosynthesis of geroprotective drugs in future research.*

Key words: *aging, bioactive substances, Caenorhabditis elegans, intestinal autofluorescence*

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СНИЖЕНИЕ НАКОПЛЕНИЯ АУТОФЛЮОРЕСЦЕНЦИИ КИШЕЧНИКА У *CAENORHABDITIS ELEGANS* ПРИ ЛЕЧЕНИИ НАТУРАЛЬНЫМИ ПРОДУКТАМИ НА РАСТИТЕЛЬНОЙ ОСНОВЕ

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РЕЗЮМЕ

Введение. *Старение – сложный процесс, связанный с постепенным снижением клеточных и физиологических функций. Геропротекторное действие 10 биологически активных веществ (БАВ) (рутин, сквален, кемпферол, биоханин А, урсоловая кислота, хлорогеновая кислота, байкалин, мангиферин, кверцетин и транскоричная кислота) и 5 неочищенных экстрактов (гинкго билоба, медуница лекарственная, шлемник байкальский, копеечник забытый и женьшень обыкновенный), выделенных из лекарственных растений Алтайского края России, были оценены на предмет их влияния на накопление кишечного аутофлуоресцентного материала (КАМ) с использованием модели Caenorhabditis elegans.*

Цель. *Кишечный аутофлуоресцентный материал способствует возрастному снижению и является неинвазивным биомаркером старения. В этом исследовании оценивалось влияние различных биоактивных веществ на снижение накопления КАМ с использованием модели C. elegans.*

Материалы и методы. *Беременные нематоды были синхронизированы, а затем посеяны в 96-луночные планшеты для развития до стадии L4. Каждый БАВ в концентрациях 200, 100, 50 и 10 мкмоль и экстракты с разбавлением в десять, сто и тысячу раз вводились в каждую лунку в 6 повторениях для каждой группы обработки. На 1-й, 5-й и 15-й дни инкубации взрослые нематоды L4 прошли спектрофлуориметрический анализ для определения влияния БАВ и экстрактов на накопление КАМ.*

Результаты. *Было обнаружено, что кверцетин, кемпферол, байкалин, мангиферин, экстракты G. biloba и P. officinalis продемонстрировали наиболее глубокое ингибирование накопления КАМ по сравнению с контролем. Примечательно, что концентрация мангиферина 10 мкмоль значительно ингибировала накопление КАМ, сопоставимое с концентрацией байкалина 200 мкмоль и кверцетина 100 мкмоль. Кроме того, сырые экстракты G. biloba и P. officinalis продемонстрировали соответственно 2,8- и 1,8-кратное снижение накопления КАМ.*

Заключение. *Накопление КАМ обратно пропорционально продолжительности жизни. Таким образом, БАВ, выявленные в этом исследовании для модуляции накопления КАМ, могут служить важными предшественниками или активными ингредиентами для фармакосинтеза геропротекторных препаратов в будущих исследованиях.*

Ключевые слова: *старение, биологически активные вещества, Caenorhabditis elegans, аутофлюоресценция кишечника*

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INTRODUCTION

By 2050, every sixth person in the globe will be over 65 years old, making up 16 % of the overall population, as opposed to 2019, when only 9 %, or every eleventh person lived past this mark [1]. Furthermore, it has been predicted that by 2050, the number of seniors (those over 65) worldwide will triple. As people age in life, there is a change in physical abilities, which may lead to unpleasant experiences, such as a decline in mental capabilities, difficulty in social interactions, or limitation in the types and quality of task execution. These transformations are manifested as one of the several age-related diseases like cardiovascular diseases, cancer, neurodegeneration (Parkinson's, Alzheimer's, and other dementia). The process of aging is intricately linked to the steady decline in physiological and cellular activities [2]. However, current research has shown that food or naturally occurring substances referred to as geroprotectors can slow down, prevent, or even reverse this age-related deterioration [3].

According to recent aging studies, bioactive compounds have exhibited enormous promises for managing even the most severe ailments and may one day replace conventional medications [4]. Nevertheless, the effects of many medicinal plant products on human are largely a mystery, despite the multitudes of research in this area. *G. biloba, P. officinalis, S. baicalensis, H. neglectum* and *P. ginseng* (or their active compounds) are some of the medicinal plants that are often explored for their geroprotective properties [5]. These plants were chosen because of their folkloric usage in the Altai region for the management of various geriatric conditions as well as their established abundance in biologically active substances. The intestinal autofluorescence material (IAM – identified by various scholars as lipofuscin, age-pigment or death fluorescence) is a non-invasive biomarker of senescence that can be monitored to evaluate the geroprotective effect of bioactive substances in animal models over time [6]. It is an intralysosomal waste material with a complex chemical composition including proteins, polyunsaturated fatty acid oxidation products, carbohydrates, and some trace elements (such as Al, Ca, Cu, Fe, Mn, and Zn) in varying concentrations [2]. In mammals and invertebrates, the build-up of IAM has been established as the most persistent and perceptible alteration throughout the aging process [2].

Although, IAM has been reported not to be biodegradable, which is further highlighted by the fact that starving cells with activated autophagy and lysosomal hydrolases are unable to break down or get rid of the pigment [7]. This could be due to the polymerization and cross linking of peptides with aldehydes that results in plastic-like structures resistant to biological degradation thereby leading to bioaccumulation over time [7]. However, there is no consensus regarding the likelihood of lysosomal IAM degeneration or its exocytosis-mediated removal. The most recognizable characteristic of IAM is its fluorescence property, which can be detected through spectrofluorimetric analysis, or laser-scanning microscopy at excitation/emission maxima of 290–420/430–650 nm [2, 8]. The intensity of colour emission changes over time (from immature pigment to mature pigment), which makes it easy to monitor [8]. Short-lived creatures like fruit flies, zebra fish, mice, rats, and nematodes accumulate the IAM at a greater rate than long-lived species thus validating their use as model animals for aging studies. This is particularly because of their highly active mitochondria, which produce more superoxide and hydrogen peroxide radicals than long-lived species [9]. These models make it easier to thoroughly research how newly created medications affect the aging process. However, the model organism *C. elegans* is gaining increasing popularity in these studies because of its numerous advantages. They include short lifespan, easy lab maintenance, transparent body for real-time imaging, advanced genetic, genomic and molecular tools and manipulations, high genetic homology with human, etc.

Autofluorescence has long been recognized as a correlate of aging in *C. elegans* [8]. Similar to mammalian cells, *C. elegans* intestinal cells frequently exhibit intracellular granules of lysosomal origin, which are the primary source of autofluorescence [10]. In this study, 10 biologically active substances (BASs) namely rutin, squalene, kaempferol, biochanin A, ursolic acid, chlorogenic acid, baicalin, mangiferin, quercetin and trans-cinnamic acid isolated from medicinal plants of Altai Region of Russia and 5 crude extracts (*G. biloba*, *P. officinalis*, *S. baicalensis*, *H. neglectum*, and *P. ginseng*) were evaluated for their capacity to delay the onset of aging by monitoring the dynamics of IAM accumulation using *C. elegans* model.

MATERIALS AND METHODS

Reagents and chemicals

Tryptone (Lot No. 1135C419), agar – European type technical grade (Lot No. 0000542402) and peptone were products of VWR Life Science AMRESCO Inc. (Russia), AppliChem GmBH (Germany), and Helicon (Russia), respectively. Tissue culture 96-well plates (TPP, Switzerland), 90 mm Petri dishes (Pertin, Russia), and other chemicals used were of analytical grade, supplied by Thermo-Fisher Scientific Inc. (Russian Federation).

Biologically active substances and crude extracts

Stock solutions of all the BASs (10 mM) and crude extracts were provided by the Laboratory of Bioassay of Natural Nutraceuticals, Kemerovo State University (Russia) and stored at 4 °C. The crude extract of *G. biloba* and *P. ginseng* were prepared by percolation in a solution of 50 % ethanol mixed with water-glycerol (1:1, v/v %); *S. baicalensis* and *H. neglectum* were extracted in a solution of 50 % ethanol and water-propylene glycol (1:1, v/v %); whereas *P. officinalis* was extracted in 50 % ethanol. Then, all extracts were diluted in sterilized deionized water in ten-, hundred- and thousand-times and used in this study. The BASs were dissolved in sterilized deionized water to arrive at the concentrations of 200 μM, 100 μM, 50 μM, and 10 μM of each BAS.

Nematode growth conditions

The nematodes *C. elegans* wildtype N2 Bristol strain was provided by the Swammerdam Institute of Life Sciences, Department of Molecular Biology and Microbial Food Safety, University of Amsterdam (Netherlands). They were seeded with 100 µL of *Escherichia coli* OP50 (Engelhardt Institute of Molecular Biology, Russia) pre-coated on nematode growth medium (NGM) plates and housed in a Binder Growth Chamber (KBWF 240, GmbH Tuttlingen, Germany) at 20 °C.

Nematodes synchronization

Adult gravid nematodes were synchronized using sodium hypochlorite solution to collect eggs, which were left overnight in S-media to produce L1 nematodes. The young nematodes were inoculated with 100 mg/mL of *Escherichia coli* OP50 and 120 µL of the *C. elegans/E. coli* OP50 solution, were dispensed in 96-well plates, where they were allowed to mature to adult L4 stage. Forty-eight hours later, an aliquot (15 µL) of 1.2 mM 5′-fluoro-2-deoxyuridine (FUDR) was added into each well. Finally, after 24 hours, 15 µL of each BASs and crude extracts at varying concentrations were added to each well to make up a total volume of 150 µL/well. The dynamics of IAM accumulation was evaluated per-treatment group on days 1, 5, and 15. All experiments were conducted with 6 repeats for each treatment condition. The nematode count per well for BASs treatment and the crude extract treatments are shown in Supplementary Tables S1 and S2 respectively.

Intestinal autofluorescence accumulation assay

The level of IAM accumulation was measured spectrofluorimetrically at excitation – emission maxima of 340–430 nm using CLARIOstar® Plus multimodal plate reader (BMG Labtech, Germany). The non-invasive measurement of IAM accumulation was first recorded 24 hours after treatment with the BASs and crude extracts after which the live nematodes were returned to their growth chamber. In the control group, the BASs or crude extracts were replaced with an equal volume of S-media. On days 5 and 15 post-administrations of the BASs and crude extracts, the measurements were repeated. Each experiment was repeated three times.

Statistical data analysis

The data obtained were expressed as the difference between the mean relative fluorescence units-RFU (Supplementary Tables S3–S8) of IAM accumulation at each day of measurement compared to the first day (Equation 1) for the mean value of six replicates per treatment group. The statistical analysis was conducted using Microsoft Office Professional Plus 2021 (Microsoft Corp., USA) and GraphPad Prism 9.0 (GraphPad Software, USA). Using Tukey's *post-hoc* test, the results were considered to be statistically significant at $p < 0.05$ and the error bars on each graph depicts the standard error of means.

$$RRFUdifference = \{MeanRFUforDay5(day15) - meanRFUforDay1\} \quad (1).$$

RESULTS

Dose-dependent mode of BASs influence on IAM accumulation

The administration of 50 µM and 100 µM of rutin significantly ($p < 0.05$) decreased the rate of IAM accumulation by 0.8- and 1.2-folds, respectively, when compared to the control (Fig. 1). In addition, the difference in RFU of IAM accumulation in *C. elegans* treated with 200 µM and 100 µM of quercetin were 1.8- and 2.1-folds, respectively (Fig. 1). Similar to rutin, the administration of 200 µM and 50 µM of kaempferol significantly ($p < 0.05$) decreased the rate of IAM accumulation when compared to the control (Fig. 1).

Furthermore, the 200 µM of baicalin and chlorogenic acid facilitated a reduction in IAM accumulation whereas the lower doses did not (Fig. 1). The treatment with squalene and biochanin A at all experimental doses did not have any significant effect in the rate of IAM accumulation when compared to the control (Fig. 2). Although, the accumulation of IAM was decreased at all doses investigated for ursolic acid, trans-cinnamic acid and mangiferin (Fig. 2), the decreases by mangiferin at all treatment doses (Fig. 2) were particularly noteworthy. Furthermore, the efficacy of kaempferol, baicalin and chlorogenic acid were only significant ($p < 0.05$) at 200 µM, whereas quercetin was more effective at 100 µM (Fig. 2). Mangiferin was the only compound to exhibit a significant ($p < 0.05$) decrease in IAM accumulation at the extremely low dose of 10 µM (Fig. 2). In addition, the IAM accumulation lowering effect of quercetin at 100 and 200 µM, kaempferol, baicalin and chlorogenic acid at 200 µM, and mangiferin at 50 and 10 µM, were evidently visible from day 5 of measurement compared to the control.

Lowering of IAM signals in the presence of medicinal plants extracts

Next, the 10-fold dilution of *G. biloba* and 1000-fold dilution of *P. officinalis* significantly ($p < 0.05$) decreased the accretion of IAM by 2.8- and 1.8-folds, respectively (Fig. 3). *S. baicalensis* at all treatment doses did not have a significant ($p < 0.05$) effect on the rate of IAM accumulation, when compared to the control (Fig. 3). In addition, *C. elegans* pretreated with the 100-fold dilution concentration of *H. neglectum* and 10-fold dilution concentration of *P. ginseng* exhibited 75 and 50 % decrease in IAM accumulation, respectively (Fig. 3).

Relative to the dynamics of IAM accumulation, the highest (10-fold dilution) concentration of *G. biloba* exhibited the most significant ($p < 0.05$) decrease in the rate of IAM accumulation while the lower doses were ineffective (Supplementary Table S8). In contrast, the lowest (1000-fold dilution) concentration of *P. officinalis* was required to produce a similar IAM lowering effect (Supplementary Table S8). However, the 10-fold dilution concentration of *P. ginseng* exhibited the most significant decrease in IAM accumulation relative to the control. *G. biloba* produced a swift effect, noticeable from day 5 whereas the effect of *P. officinalis* was not evident until day 15 (Fig. 3).

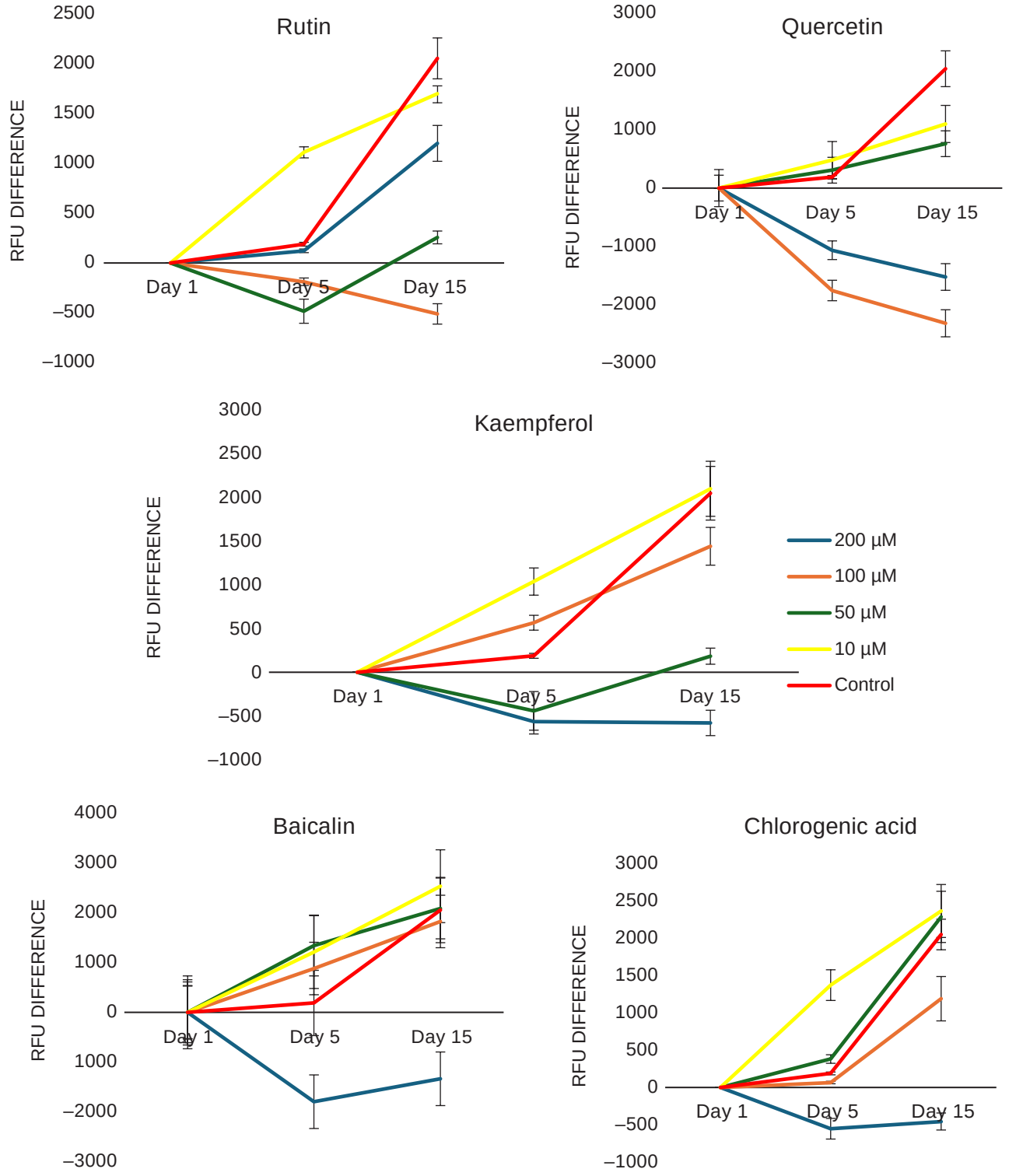

FIG. 1.

Effect of rutin, quercetin, kaempferol, baicalin, and chlorogenic acid on IAM in C. elegans. Rutin, quercetin, kaempferol, baicalin, and chlorogenic acid were added to C. elegans in each well of 96-well plate in concentrations 10 μM, 50 μM, 100 μM, and 200 μM. The IAM was measured on days 1, 5, and 15 after treatment with BASs. The IAM accumulation results were presented as a difference value between the mean RFU of 6 replicates on days 5 and 15 of measurement, compared to the RFU value on day 1.

Comparison of the inhibitory effect of the most effective BASs and extracts during *C. elegans* adulthood

The rate of IAM inhibition exhibited by the 200 μM of quercetin, kaempferol, baicalin, and mangiferin on day 15 of adulthood were 19.94 %, 18.88 %, 20.60 %, and 43.41 %, respectively, when compared to the control (Fig. 4). In addition, the crude extracts of *G. biloba* and *P. ginseng* exhibited 16.21 and 24.12 % inhibition of IAM accumulation respectively (Fig. 4).

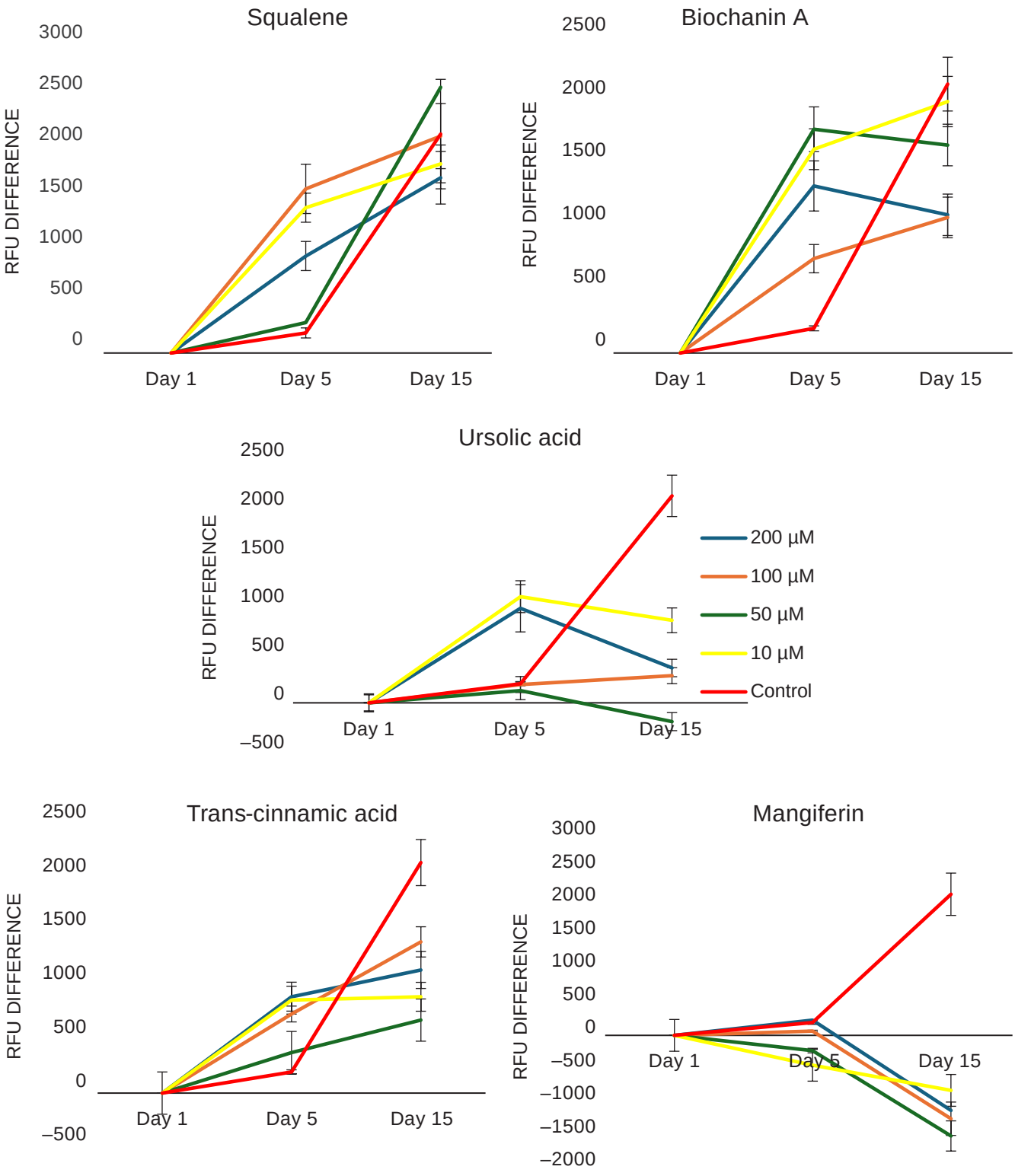

FIG. 2.

Effect of squalene, biochanin A, ursolic acid, trans-cinnamic acid, and mangiferin on IAM accumulation in C. elegans. Squalene, biochanin A, ursolic acid, trans-cinnamic acid, and mangiferin were added to C. elegans in each well of 96-well plate in concentrations 10 μM, 50 μM, 100 μM, and 200 μM. The IAM were measured on days 1, 5, and 15 after treatment with BASs. The IAM accumulation results were presented as a difference value between the mean RFU of 6 replicates on days 5 and 15 of measurement, compared to day 1 value.

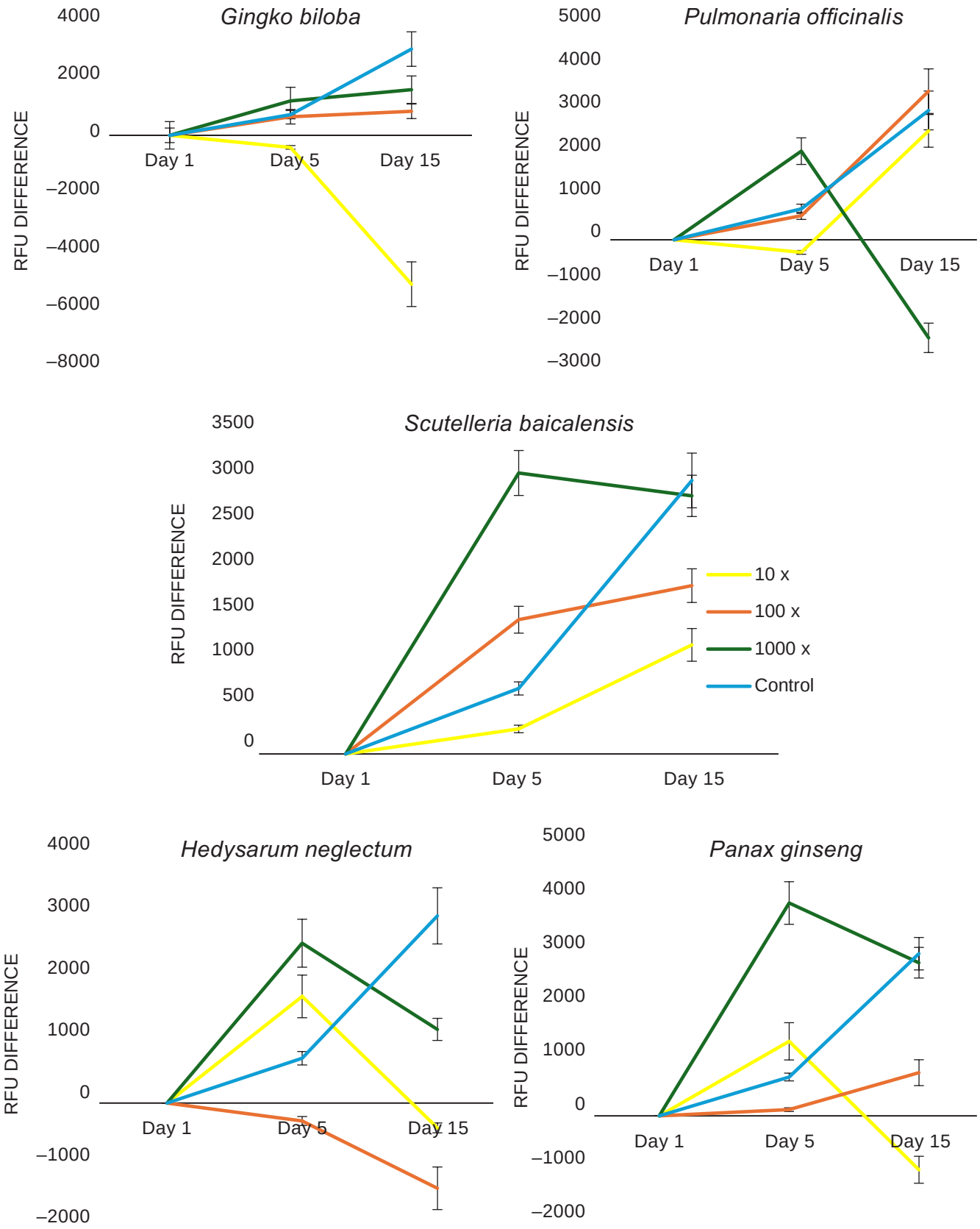

FIG. 3.

Effect of the crude extracts on IAM accumulation in C. elegans. The result of IAM accumulation was presented as a difference between day 1 and days 5 or 15 after treatment of nematodes with 10-, 100- and 1000-fold dilution concentrations of extracts isolated from G. biloba, P. officinalis, S. baicalensis, H. neglectum, and P. ginseng plants. The data represents the mean ± standard error of means for six replicates and are considered to be statistically significant at $p < 0.05$.

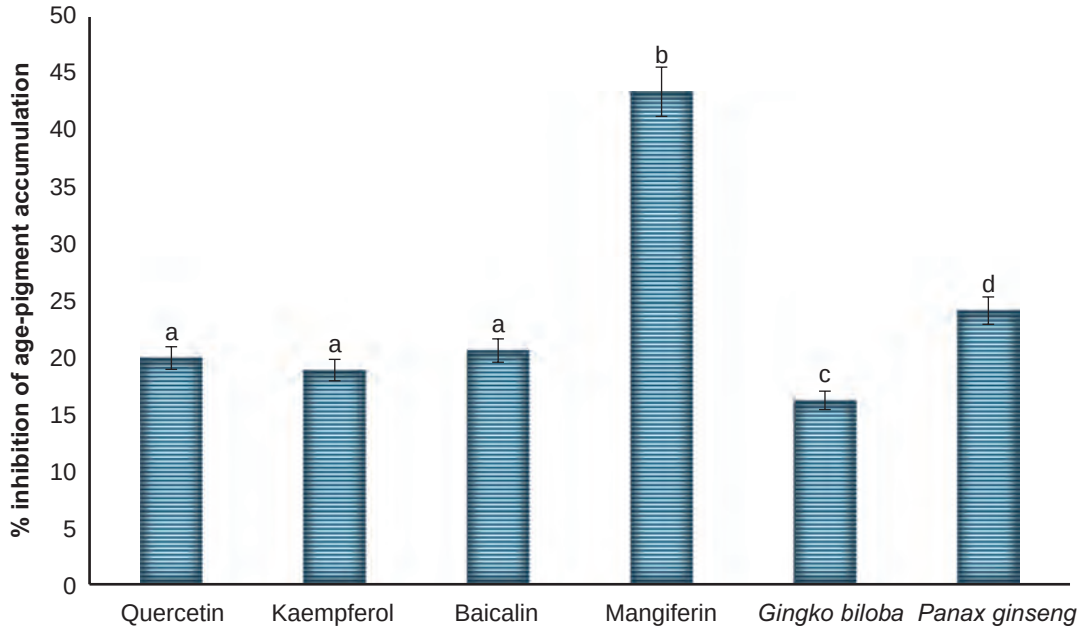

FIG. 4.

Comparison of the percentage inhibitory effect of the most efficacious BASs and extracts on age-pigment accumulation in C. elegans body on day 15 of treatment. The percentage inhibition of IAM accumulation by BASs or extracts from medicinal plants were computed relative to the control group of nematodes at the same conditions on day 15 of treatment.

DISCUSSION

Several research have shown that plant-derived nutraceuticals can increase the average lifespan of *C. elegans* [11, 12]. Also, experimental data suggests that the accretion of IAM which is inversely proportional to longevity can be modulated [13]. A cell culture experiment revealed that IAM formation was hampered by exposure to iron chelators, antioxidants and 8 % oxygen medium. In contrast, the production rate of IAM was accelerated by oxidative stressors (such as UV lights, free radicals and heat), as well as the exposure to extremely low-molecular-weight iron compounds and 40 % oxygen. A steady increase in the intracellular build-up of IAM has been reported to proliferate oxidative injury in cells via the disruption of lysosomal activity, lysosomal hemochromatosis, causing a decline in the antioxidant defense system, dysfunctional mitochondria and cell death [2]. Consequently, any substance that can slow down the rate of accretion of the IAM would make a good therapeutic option for healthy aging.

The BASs and plant extracts adopted in this study are potential geroprotectors with their influence on longevity, antioxidant, and other age-related ailments already established in open scientific literature. For instance, quercetin was reported to exhibit a significant extension of lifespan, stimulate motility in heat-stressed and aged nematodes *via* the modulation of *hsf-1* activity, p38-MAPK and IIS pathways [14]. Baicalein has been shown to influence longevity and stress tolerance in *C. elegans* via *skn-1*, whereas it does not affect *daf-16* [15]. Chlorogenic acid and its isomers (4-caffeoylquinic acid and 5-caffeoylquinic acid) acted on the IIS pathway's upstream of *akt*, and then its life-extending and anti-aging properties were primarily mediated through *daf-16* and its downstream stress factors, *hif-1*, *skn-1*, and *hsf-1* [16]. The life extension and oxidative stress tolerance effect of trans-cinnamic acid was also reported by A.M. Fedorova et al. [17]. *G. biloba* has been reported to prevent mitochondrial dysfunction through the suppression of caspase-9 activation, mitochondrial ROS generation as well as the expression of toll-like receptors thereby mitigating cell necrosis [18]. Similarly, various extracts of ginseng have been reported to sustain healthy aging and lifespan in *C. elegans* [19].

The aggregation of the aging marker IAM increases with time which can be aggressive in a number of age-related debilitating diseases, thus leading to cellular and organofunctional deterioration [2]. Hence, our research was designed to investigate the influence of our BASs and crude extracts on the dynamics of IAM accumulation.

The results of our study suggests that the BASs – quercetin, kaempferol, baicalin and mangiferin as well as the crude extracts of *G. biloba* and *P. officinalis* exhibited the most profound effect in the lowering of IAM accumulation albeit at varying concentrations. In contrast, our study could not find a substantial amount of evidence to support the effectiveness of rutin and squalene as they stimulated the process of IAM production while biochanin A was rather inconclusive.

Furthermore, during aging, there is an unwanted decline in the rate of auto-phagocytosis, proteolysis, lysosomal degradation, mitochondrial function, exocytosis, and lipid metabolism all of which partly or in combination promotes the intracellular aggregation of the IAM [20]. Quercetin, a prominent food antioxidant, was able to mitigate the accretion of IAM by 2.1-folds which was consistent with the findings by A. Kampkötter et al. [21]. The amelio-

rative effect of quercetin on mitochondrial function and lipid metabolism has been well established as it can restore mitochondrial membrane potential, scavenge reactive oxygen species, and stimulate AMP-activated protein kinase (AMPK) activity [22]. Also, quercetin has been attributed with the restoration of lysosomal function and autophagy flux in pancreatic β-cells through the modulation of lysosomal membrane permeability [23]. Thus, the reversal of lysosomal dysfunction, improvement of lipid metabolism, stimulation of mitochondrial function and autophagocytosis could be some of the plausible mechanisms responsible for the effective reduction in IAM accumulation exhibited by quercetin as early as day 5. The steady decline up to day 15 suggests that the rate of elimination exceeds the rate of accumulation, resulting in a net offset of the IAM. Both *in vivo* and *in vitro* studies suggests that the activation of AMPK facilitates lipolysis and suppresses lipid biosynthesis. *Scutellaria baicalensis* and its active constituent baicalin play integral role in the prevention and management of metabolic syndrome through the upregulation of the AMPK-facilitated modulation of glucose and lipid metabolism [24]. Similarly, kaempferol in combination with cinnamaldehyde ameliorated lipid metabolism disorders via the activation of AMPK. Thus, kaempferol and baicalin might have acted to subjugate the accretion of IAM via the regulation of lipid metabolism. Also, baicalin exhibits iron-chelating property thus, precluding iron overloading which promotes IAM production [25]. The IAM accumulation mitigatory effect exhibited by kaempferol in our study corroborates the findings by A. Kampkötter et al. [21] where treatment with fisetin and kaempferol led to a reduction in IAM accumulation.

The tremendous health benefit of mangiferin has been reported, ranging from antioxidative, antiaging, antidiabetic, immunomodulatory, antiviral amongst others [26]. Mangiferin shields the cell from oxidative stress through the expression of antioxidant enzymes catalase, superoxide dismutase, glutathione peroxidase and mitigates lipid peroxidation [26]. Also, mangiferin stimulates the production of proteins such as cytochrome C oxidase subunit 1, important for mitochondrial biogenesis and suppresses the production of proteins such as acetyl-coA carboxylase 1, integral for lipogenesis [27]. Hence, the protective effect of mangiferin towards the aggregation of IAM particularly at the lowest concentration (10 μM) implies that it must have acted through multiple mechanisms. Also, it can be adduced from our findings that a trace amount is enough to stimulate the myriads of molecular interventions critical for the inhibition of IAM biogenesis including the restoration of AMPK-mediated autophaghy, oxidative balance, lysosomal and mitochondrial function, modulation of lipid and carbohydrate metabolism [22]. The incapability of rutin (a glycosylated variant of quercetin – quercetin-3-O-rutinoside) to delay the accumulation of IAM has also been reported by A. Kampkötter et al. [21]. This could be attributed to the absence of more reactive centers in rutin, thus reducing its antioxidant potential, when compared to its aglycone variant quercetin. However, this is in contrast to our findings where the 100 μM of rutin significantly reduced the rate of IAM accumulation.

Also, the ineffectiveness of squalene (a long chain fatty acid-$C_{30}H_{50}$ and precursor for cholesterol synthesis) could be due to the absence of the cholesterol-synthesis branch of the mevalonate pathway in *C. elegans* [28]. This could lead to bioaccumulation and probably serve as lipid source for IAM production, hence the increased production reported in this study. Unlike rutin and squalene, the geroprotective effect of biochanin A is still unclear. However, the solubility and bioavailability of biochanin A has been reported and its ineffectiveness in the mitigation of IAM accumulation could simply be attributed to its poor absorption by the nematodes to elicit its biological activity.

Ursolic acid and trans-cinnamic acid were able to slow down the rate of IAM accumulation at all doses investigated. However, their effects were not in folds which might imply poor pharmacokinetics between the drug(s) and organism, or the dose range adopted in this study is outside the optimal dosage required to stimulate pharmacological response comparable with quercetin and mangiferin.

The 10-fold dilution concentration of *G. biloba* and 1000-fold dilution concentration of *P. ginseng* exhibited the most profound reduction in IAM accumulation, whereas *P. officinalis*, *S. baicalensis* and *H. neglectum* exhibited a moderate reduction which can be attributed to their constituent BASs. Quercetin and kaempferol were isolated from *G. biloba* while mangiferin was isolated from *H. neglectum*. Furthermore, age-related deterioration involves multiple pathologies that act in synchronization to facilitate aging. As such, the multitudes of BASs present in the crude extract could exert antiaging effect via pharmacodynamic synergy with various compounds acting on different receptor targets of the aging pathways [29]. Thus, the swift and profound effect of the crude extracts of *G. biloba* reported in this study can be attributed to the abundance and potential synergistic effect of these BASs towards the repression of IAM production. A similar effect was not observed in *H. neglectum* treatment group, probably due to the presence of other impurities that might have acted as antagonist for the bioactive function of mangiferin. Our findings on the effect of *P. ginseng* on IAM accumulation are consistent with current available literature [19]. Ginsenoside, a bioactive component of *P. ginseng* has been reported to extend lifespan and reduce IAM accumulation via the modulation of lipid metabolism and activation of the stress response biomolecules [30]. The rate of IAM clearance was also facilitated in *C. elegans* treated with the 1000-fold dilution concentration of *P. officinalis*. This can be attributed to the mitigation of oxidative stress via the removal of mitochondrial ROS which culminates in enhanced mitochondrial function.

CONCLUSION

The intestinal autofluorescence material is not only a hallmark of aging but also a facilitator of age-related deterioration; thus, any substance capable of clearance and/or slow down the rate of accretion of IAM will constitute an important supplement towards healthy aging. Our finding has identified baicalin, quercetin, kaempferol, and mangi-

ferin as potential geroprotectors that could be subjected to further studies towards the realization of the UN decade goal of healthy aging. Their probable mechanism of clearance includes the enhancement of intra-lysosomal degradation, autophagocytosis, mitochondrial function and lipid metabolism. In addition, *G. biloba* and *P. officinalis* can be considered as an important source for potential geroprotector development in future research.

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Conflicts of interest

The authors declare no conflict of interest.

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Ethical Clearance Statement

According to the Caenorhabditis Genetics Centre, concerns about ethics are absent from *C. elegans* experiments. However, all the methods adopted during this study, which received approval on December 23, 2021, conformed to the Guide for the Care and Use of Laboratory Animals, published by the United States National Institutes of Health (Publication No. 85-23, revised 1996) and approved by the Moscow Institute of Physics and Technology Life Science Center Provisional Animal Care and Research Procedures Committees, Protocol No. A2-2012-09-02.

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Author's contribution

Saoban S. Salimon – performing the experiments; data collecting and analysis; writing the original draft.

Elena I. Marusich – design of the study and methodology; reviewing and editing the manuscript.

Sergey V. Leonov – administration of the project, finance and supplies support; editing the manuscript.

Margarita V. Pustovalova – procuring funding; editing the manuscript.