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Original Research

Heptoprotective Role of *Artemisia scoparia* Waldst. and Kit Against CCl_4-induced Toxicity in Rats

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Abstract

In this study, the methanol extract of *Artemisia scoparia* was evaluated for its protective potential against carbon tetrachloride (CCl_4)-induced hepatic toxicity. Seven groups of mature albino rats were used in the course of the experiment and each group was treated with specific doses of plant extract and CCl_4. Silymarin was used as a standard protective drug. The results of the experiment revealed that *Artemisia scoparia* plant extract was successful in fighting CCl_4 toxicity as it clearly reduced the elevated levels of liver serum markers (alkaline phosphatase and alkaline aminotransferase), lipid peroxidation, nitrite content, and H_2O_2 on one side while enhancing the levels of antioxidant enzymes (catalase, peroxidase,

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superoxide dismutase, glutathione-s-transferase, γ-Glutamyltranspeptidase, and glutathione reductase) and protein content. It also protected DNA from the damaging effects of CCl_4. The findings of this study demonstrate that *Artemisia scoparia* plant extract plays a significant role in preventing the hepatic damages instigated with CCl_4 and can be used as a protective agent against oxidative stress-associated disorders.

Keywords: *Artemisia scoparia*, hepatoprotective, rats, folk use

Introduction

Free radicals are produced physiologically inside the body and are eliminated by antioxidant enzymes. Whenever the production and removal of free radicals becomes imbalanced, they accumulate in the body and become harmful to the organs [1-2]. Different plants are a massive supply of bioactive constituents involved in the scavenging of oxidation-prompting radicals [3-4].

The natural antioxidants work as a shelter against the assaults of free radicals that can be the cause of diverse irreversible harm to the cell. The therapeutic potential of medicinal plants is attributed to their secondary metabolites. The scavenging of free radicals by the plant-derived product may offer a natural alternative approach to combat stress-induced tissue damage [5].

Artemisia scoparia Walds. and Kit (red stem wormwood: jhahoo) belongs to the Asteraceae family (Compositae), commonly known as red stem wormwood and locally called jhahoo or jaukay. It grows in the summer season, in the sandy soil of barren areas, after rainfall, beside side roads, on stony ground, and in wastelands and rural tracks from 450 to 2,200 m altitude. It is a perennial and slightly aromatic herb [6]. Traditionally *A. scoparia* is used for earache. Its smoke is believed to be an excellent remedy against burn conditions and also is used as a purgative [7]. This plant is rich in volatile oils that display a broad spectrum of biological activities and has extensive applications in medicine. It has been known for its antipyretic, anticholesterolemic, antiseptic, antibacterial, cholagogue, diuretic, and vasodilator properties and also for the treatment of gall bladder, hepatitis, inflammation, and jaundice [8]. Ibrar and Hussain [9] reported that local healers utilize the aerial parts of *A. scoparia*in kidney and liver disorders.

Carbon tetrachloride (CCl_4) is a well-known toxic chemical that enhances free radical generation by decreasing the levels of antioxidant enzymes like catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and glutathione peroxidase (GSX), and enhances the levels of thiobarbituric acid reactive substances (TBARS). CCl_4 also is involved in the elevation of serum membrane marker enzymes, i.e., alkaline phosphatase (ALP), amino transaminase (AST), gamma-glutamyltranspeptidase (γ-GT), and alanine transaminase (ALT), and lowers albumin and creatinine clearance, which indicate abnormality of two major organs: liver and kidney [10]. This toxic chemical also damages DNA and causes breaks in it through its oxidative stress [11]. In contrast, the medicinal plants reversed the harmful effects of CCl_4. Khan [12] studied the protective effects of *Launaeacprocumbens* against $KBrO_3$ toxicity and reported the effectiveness of plant extract against toxicity. Guha and Venkatadri [13] reported that some marvelous medicinal plants have the ability to protect DNA.

In this study we have evaluated the protective effects of the methanol extract of *A. scoparia* aerial parts against the oxidative assault induced with CCl_4 in hepatic tissues. In this regard comet assay and the activity level of various antioxidant enzymes of hepatic tissues along with a biochemical analysis of serum was performed to demonstrate the protective potential of *A. scoparia* in hepatic tissues.

Materials and Methods

Plant Collection and Extract Preparation

The plant was collected in spring 2013 and authenticated by Dr. Mushaq at Quaid-i-Azam University in Pakistan. The plant was dried in shade and ground to a mesh size. 2 kg powder of plant material was soaked in 5 L of crude methanol for seven days. Then filtrate was obtained and evaporated under reduced pressure and stored at 4ºC for future use.

Animal Treatment

Protocol of the present study regarding laboratory animal food and care was approved by the ethical committee of Quaid-i-Azam University. The study was conducted with 42 mature male albino rats in a primate animal facility of the university. All healthy animals of weight 150-200 g were kept under standard conditions and standard diet and divided into seven groups randomly (six animals in each group). The first group was untreated and taken as control. Group 2 was treated with 10% DMSO in olive oil (1 mL/kg). Group 3 was treated with 30% CCl_4. Group 4 was treated with silymarin (100 mg/ml) and 30% CCl_4. Group 5 was treated with a low dose of plant extract (150 mg/kg) and 30% CCl_4. Group 6 was treated with a high dose of plant extract (300 mg/kg) and 30% CCl_4. Group 7 was treated with plant extract (300 mg/kg) alone. Animals were treated on an alternate day for 30 days. After the experimental period (30 days), the animals were sacrificed. First, the blood was taken and then liver excised. Serum was obtained by centrifuging the blood sample and storage at -80ºC. The excised organ was washed in ice-cold saline solution and further treated according to future need. Some part of the organ was treated with liquid nitrogen and kept at -80ºC for enzymatic and comet assays.

Homogenate Preparation

Tissues of the liver (100 mg) were weighed and homogenized in 1 ml 100mM potassium phosphate buffers. The buffer was mixed with 1 mM EDTA. Homogenate was centrifuged at a speed of 10,000 rpm at 4ºC for 30 mins and the supernatant was collected and preserved at -80ºC for further use.

Serum Markers

Two main liver functional markers (i.e., ALP, ALT, and serum albumin levels) were measured by using standard AMP kits (Roche Diagnostics GmbH, Mannheim, Germany).

Hydrogen Peroxide (H_2O_2) Assay

This assay was done by the protocol of [14]. Horseradish peroxidase enzyme was used, which was mediated by H_2O_2. The sample absorbance was documented at 610 nm against reagent as a blank. The H_2O_2 concentration was noted as nM H_2O_2/min/mg.

Estimating Lipid Peroxidation Assay (TBARS/LPO)

The lipid peroxidation or TBARS estimation was done by the method of Kanter et al. [15] with some alterations. Ascorbic acid and ferric chloride were added to the reaction mixture and trichloroacetic acid was used to stop the reaction. Optical density was recorded at 535 nm, which gave information about TBARS generated in the test sample. Lipid peroxidation activity expressed as TBARS formed/min/mg protein.

Protein Estimation

The protein estimation of liver tissue supernatant was assayed by Naz [16] protocol. Bovine serum albumin (BSA) curve was used to figure out the concentration of serum proteins in the sample.

Nitrite Assay

We followed the methodology of Khan [17] for nitrite activity estimation. $ZnSO_4$ was used for deprotonation. Griess reagent was used, and alteration in solution color was noted at 540 nm.

Antioxidant Enzyme Evaluation

Catalase and Peroxidase activities were performed by the protocol of Shah and Khan [4]. The results obtained were documented in units/mg protein with minor modification. H_2O_2 was used in CAT assay and absorbance was noted at 240 nm, while in POD assay the substrate was guaiacol and absorbance was noted at 470 nm. In both assays, one unit activity defines by absorbance change of 0.01 as units/min. In the case of superoxide dismutase (SOD) the protocol of Kakkar and Das [18] was followed and phenazinemethosulphate and sodium pyrophosphate were used. The absorbance was measured at 560 nm. The results obtained were documented in units/mg protein.

Glutathione-S-transferase (GST) Assay

GST for liver cells was done by the protocol of Shah and Khan [19]. Reduced glutathione and CDNB was used in the course of the assay. The absorbance of the test sample was recorded at a wavelength of 340 nm. With the help of the molar extinction coefficient (9.6×103/M/cm), enzymatic activity (GST) was expressed as nM CDNB conjugate formed/min/mg protein.

γ-Glutamyltranspeptidase (γ-GT) Assay

The activity of γ-glutamyltranspeptidase was assayed by adopting the scheme of Sajid and Khan [20]. According to this scheme, glutamylnitroanilide was utilized as the substrate. Optical density was assessed at 405 nm through a spectrophotometer. Using a molar extinction coefficient of 1.75×10^3/mol/cm, the activity of γ-GT was determined as Nm nitro-aniline formed per min per mg protein.

Reduced Glutathione (GSH) Assay

For this assay, the protocol of Ahmad and Khan [21] was followed, which involved the use of sulfosalicylic acid and DTNB. The reaction mixture showed the yellow colored complex immediately at 412 nm. The activity of the enzyme is expressed as µM GSH/g of tissue.

Comet Assay

For accomplishing the comet assay we followed the protocol of Dhawan and Bajpayee [22] with some modifications. Electrophoresis of microgel slides was accomplished under alkaline conditions and slides were observed with the help of a fluorescent microscope.

Statistical Analysis

All parameters except comet assay were statistically analyzed by using computer software statistix 8.1. The parameters were analyzed through a Graphpad prism (version 5). The one-way analysis of difference was measured by one-way ANOVA test at probability level 0.01%, and results are documented as the means ±standard deviation of triplicate analyses.

Table 1. Effect of methanol extract of *A. scoparia* on liver serum markers and albumin levels.

Treatment	Albumin (mg/ml)	ALT (U/I)	ALP (U/I)
Control	3.50±0.40c	32.14±1.72e	63.11±1.85e
Olive oil	3.46±0.35c	31.21±1.56e	61.58±1.20e
CCl_4 (1 mL/kg b.w)	6.33±0.55a	68.80±2.03a	122.84±2.94a
CCl_4+silymarin	5.40±0.55ab	35.75±1.03de	75.79±1.14c
CCl_4+Extract (150 mg/kg b.w)	4.60±0.50bc	54.95±1.17b	92.52±1.89b
CCl_4+Extract (300 mg/kg b.w)	4.13±0.55bc	43.74±1.54c	69.51±1.00d
Extract (300 mg/kg b.w)	3.60±0.30c	37.88±0.94d	62.73±1.32e

Mean±SD (n = 6); letters a-e indicate level of significance at $p<0.01$

Results

Protective Potential of Methanol Extract of *A. scoparia* on Liver Serum Markers

CCl_4 treatment significantly enhanced the levels of both liver markers as compared to their levels in the control group's rats. In contrast, the co-treatment of extract with CCl_4 lowered the levels of liver markers dose-dependently and treating high dose alone exhibited quite safe effects. For ALT the level of serum markers was slightly higher than the control group level, but in the case of ALP the level of serum markers of ASME-treated rats were almost equal to the control group rats. In the case of albumin, the CCl_4 was responsible for the rise in serum albumin level in comparison with the normal level. The methanol extract of *A. scoparia* and silymarin successfully reduced the rise in albumin level. The plant extract showed its protective effects in a dose-dependent fashion, and a high concentration of extract was found to be more effective (Table 1).

Protective Influence of Methanol Extract of *A. scoparia* on Liver Protein, TBARS, H_2O_2, and Nitrite Content

A significant decrease in protein level of CCl_4-treated rats is clear evidence of toxicity induction. Co-treatment of extract successfully reduced the toxicity of CCl_4 and restored the protein level dose dependently. CCl_4 treatment boosted the concentration of TBARS, H_2O_2, and nitrite content, while the co-induction of extract successfully reversed the toxic effects and restored the normal levels. The high dose of the extract was effective in parallel to standard silymarin in the prevention of lipid peroxidation, in the H_2O_2 assay and nitrite content assessment (Table 2).

Protective Effect of Methanol Extract of *A. scoparia* on Liver Antioxidant Enzymes and GSH Profile

The CCl_4 treatment was responsible for the clear decrease in levels of CAT, POD, and SOD of liver tissue. The extract administration reversed the elevated level of these enzymes. CCl_4 treatment also markedly decreased the levels of GST and GSH as compared to

Table 2. Effect of methanol extract of *A. scoparia* on hepatic levels of protein, TBARS, H_2O_2, and nitrite content.

Treatment	TBARS (nM/min/mg protein)	Nitrite (μM/mL)	H_2O_2 (μg/mg tissue)	Protein (μg/mg tissue)
Control	35.36±1.55bc	48.43±1.15c	6.69±0.51d	2.43±0.35a
Olive oil	36.00±1.41bc	49.66±1.00bc	7.24±0.39cd	2.20±0.30a
CCl_4 (1 mL/kg)	82.23±3.65a	91.93±4.15a	17.70±0.73a	0.72±0.10c
CCl_4+silymarin	34.93±1.55b	47.60±1.15c	8.48±0.43cd	1.70±0.30b
CCl_4+ Extract (150 mg/kg)	31.10±1.45c	52.60±2.10b	11.92±0.66b	1.60±0.31b
CCl_4+ Extract (300 mg/kg)	34.56±1.10bc	50.46±1.16bc	9.95±1.16bc	1.80±0.30b
Extract (300 mg/kg)	36.63±1.10b	49.56±1.12bc	7.43±0.05cd	2.06±0.25a

Mean±SD (n = 6); letters a-d indicate level of significance at $p<0.01$

Table 3. Effect of methanol extract of *A. scoparia* on hepatic antioxidant enzymes.

Treatment	CAT (U/min)	POD (U/min)	SOD (U/mg protein)	GST (nM/min/mg protein)	GSH (nM/min/mg protein)	γ-GT (nM/min/mg protein)
Control	4.6±0.3a	11.56±1.05a	5.2±0.4a	274.00±3.4a	48.78±1.16a	1.16±0.04cd
Olive oil	4.5±0.3a	9.46±0.75ab	5.2±0.4a	254.57±4.9b	48.19±1.00a	1.15±0.04cd
CCL_4 (1 mL/kg b.w)	1.5±0.2c	2.70±0.40c	1.2±0.3c	109.60±3.0c	14.70±1.02d	3.28±0.03a
CCL_4 (1 mL/kgb.w) +silymarin	4.5±0.4ab	11.40±1.00a	4.7±0.4a	270.40±3.9a	46.45±1.05a	1.32±0.04b
CCL_4+ Extract (150 mg/kg b.w)	3.1±0.5b	7.90±0.80b	2.9±0.4b	254.90±4.0b	28.97±1.11c	1.22±0.05bc
CCL_4+ Extract (300 mg/kg b.w)	3.5±0.5b	10.26±1.05ab	4.4±0.4a	267.97±3.4a	41.74±1.00b	1.21±0.03bc
Extract (300 mg/kg b.w)	4.0±0.5ab	8.23±0.86b	4.7±0.5a	271.10±3.4a	45.74±1.16a	1.05±0.04d

Mean±SD (n = 6); letters a-c indicate level of significance at $p<0.01$

the control group. Extract co-treatment ameliorated the toxicity of CCl_4 in a dose-dependent manner. In the case of γ-GT, the CCl_4 administration was responsible for the increase in the level of γ-GT. This toxic effect was reduced by extract treatment and a higher concentration of extract was more effective in toxicity prevention (Table 3).

The Role of *A. scoparia* Methanol Extract in DNA Protection of Liver Cells

The results revealed that CCl_4 induced the damaging effects on DNA of liver cells as the tail length of these cells' DNA was greater than the control group animal cells' DNA, and head length of CCl_4-treated cells was reduced as compared to normal ones (Table 4). Two other parameters of DNA comet length and tail moment were also increased in these cells, while these negative effects were tremendously decreased by methanol extract of *A. scoparia* (Fig. 1). The protective effects were dose-dependent and a high dose of plant extract was found to be more protective to DNA.

Discussion

Free radicals are considered to be involved in DNA damage, lipid peroxidation, and protein injuries, leading to acute or chronic hepatic disorders. Toxic manifestations of the reactive species can be ameliorated by taking a diet rich in antioxidant metabolites. Aerial parts of *A. scoparia* are composed of diverse metabolites having antioxidant abilities [23-24].

Carbon tetrachloride is considered to be a dangerous hepatotoxin [25]. It swings the oxidant-antioxidant balance toward negative by agitating the antioxidant enzyme defensive system. Cytochrome P-450 metabolizes CCl_4, which generates free radicals. These free radicals afterward instigate endoplasmic reticulum lipid peroxidation and start a prolonged chain reaction. Due to this oxidative stress, massive damage to proteins, DNA, and cellular proteins occurs [26].

The hepatoprotective effect of methanol extract of *A.scoparia* was investigated by analyzing different biochemical and molecular parameters. The liver serum markers' biochemical parameters were assessed. Rats treated with CCl_4 exhibited a great degree of damage and

Table 4. Effects of methanol extract of *A. scoparia* on DNA tail and head length of liver cells.

Treatment	Tail length (μM)	%DNA in tail	Tail moment	Head length (μM)	% DNA in head	Comet length (μM)
Control	6.0±1.1	10.5±1.7	0.62±0.5	26.5±0.9	90.6±2.5	32.5±1.7
Vehicle	5.8±1.0	10.0±1.9	0.66±0.6	24.5±0.6	88.8±2.3	33.6±1.8
CCl_4 (1 mL/kg)	40.8±1.7a***	26.2±2.6a***	2.89±0.4a***	15.5±1.7a*	73.8±2.6a**	58.3±1.9a***
Silymarin	5.6±1.1	10.6±1.1	0.65±0.4	25.6±1.8	87.9±2.5	32.8±1.7
CCl_4+ Extract (150 mg/kg)	16.5±0.9a**	5.9±1.0b***	1.15±0.6a**	18.0±3.2b**	84.1±1.0b*	34.5±3.5b**
CCl_4+ Extract (300 mg/kg)	6.9±1.1b**c*	8.8±2.2b***	0.71±0.1c*	23.0±1.29	88.5±1.4	27.8±0.8b***
Extract (300 mg/kg)	9.8±1.7b**c*	9.5±0.5b***c*	0.85±0.1a*c*	24.3±0.3	89.6±0.5	33.0±2.4b**

Mean ±SD (n = 6); *level of significance at $p<0.01$, *-0.01, **-0.001, ***-0.0001; Control vs. CCl_4-a, CCl_4vs all treated groups-b, Low dose vs. high dose and extract=c

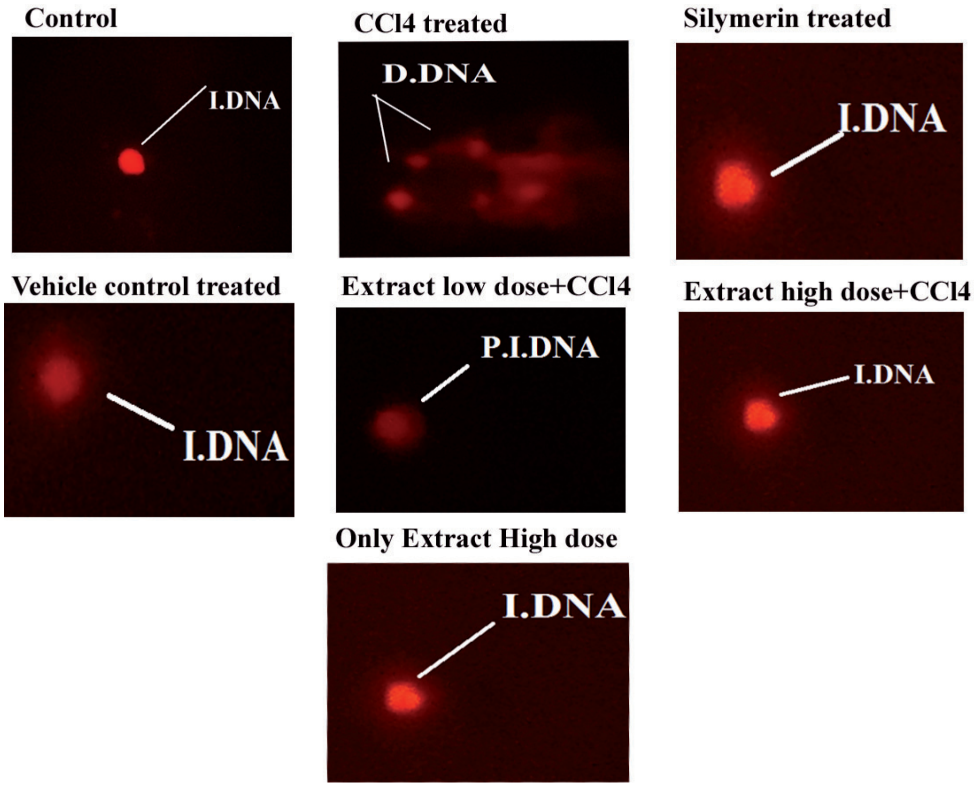

Fig. 1. Microphotographs of DNA of liver cells of different groups after treatment with methanol extract of *A. scoparia*. DNA is intact DNA, D.DNA is damaged DNA, and P.I.DNA is partially intact DNA.

enhanced levels of liver serum markers ALT and ALP. The serum levels of control group rats were correctly in the normal range and rats of the vehicle group have values close to the normal range. Silymarin exhibited great protection against toxicity as the values of silymarin-treated rats were also closed to normal values. The plant extract showed its protective capacity according to its dose concentration. The low dose (150 mg/kgb.w) was less protective while the protective effects of the high dose (300 mg/kg b.w) were highly comparable to silymarin.

CCl_4 also revealed its toxic effects by changing the levels of antioxidant enzymes, GSH, TBARS, H_2O_2, tissue protein, nitrite content, and some other biochemical parameters. The normal values were observed in control group rats, and vehicle group values were not much different. Silymarin administration decreased the CCl_4 effects and showed the values in the range of control values. The low dose of plant extract was found to be less effective at curing CCl_4 toxicity, while high doses exhibited the protective potential almost equal to silymarin.

The hepatoprotective potential of *A.scoparia* was also was determined on a molecular level through comet assay. The results of this assay proved that CCl_4 is toxic to cellular DNA. Cells of rat livers treated with this toxic reagent showed increased tail length and movement, decreased the head length, and increased comet length, which indicate damage. The DNA of control group liver cells exhibited the normal length and percentage of all parameters.

The oxidative stress generated by CCl_4 was greatly reduced by methanol extract of *A. scoparia* at all levels. The high protection capacity of this plant may be due to the presence of phenolic compounds. The same piece of work was done by Praveen and Dharmaraj [27] on assessing the hepatoprotective activity of different fractions of *Scopariadulcis* L. against CCl_4-induced toxicity in the liver of mice. They evaluated the plant's hepatoprotective potential by checking the levels of liver markers ALT, AST, and ALP, plus total proteins in serum, glycogen, lipid peroxides, and antioxidant enzyme levels in liver tissue homogenate. Results proved that CCl_4-induced toxic effects on liver serum markers, antioxidant enzyme levels, and histology of liver and these toxic effects were inverted by extract induction at a dose of 800 mg/kg b.w. On the basis of these results they concluded that the high potential as an antioxidant is due to the high content of terpenoids. The present investigation was carried out to demonstrate the hepatoprotective effects of *A.scoparia* extract against CCl_4-mediated renal oxidative trauma. The defensive outcome of *A.scoparia* was evaluated by estimating the serum marker levels and by measuring activity levels of antioxidant enzymes in hepatic tissues. Furthermore, the levels of GSH, TBARS, nitrite, and H_2O_2 were determined in hepatic tissues along with DNA damage.

We performed comet assay in order to appraise the DNA damage induced by reactive species in hepatic tissues. Comet assay is a responsive and adaptable technique that deciphers the DNA strand breakage at the single cell level [14, 28]. In the current study a significant increase in tail movement, tail length, head length, comet length, and percentage of DNA in the tail were recorded with the CCl_4 administration in hepatic cells of threat. In our results, the long tail length of comet reveals the high extent of DNA damage in CCl_4-treated hepatic cells of rats. Comet tail length is investigative of DNA fragmentation in any cell variety studied by comet assay. The altered comet parameters were reversed toward the control level by the co-administration of extract, and the protective effect on DNA was more pronounced at the higher dose of *A. scoparia*. These results suggest that *A. scoparia* is a worthy candidate to inhibit the DNA damage in hepatic tissues.

Conclusion

Our study suggests that *A. scoparia* has the ability to ameliorate the CCl_4-provoked hepatic injuries and has restored the serum markers, DNA damages, and levels of enzymatic activity. The protective effects of *A. scoparia* might possibly be associated with its antioxidant properties.

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