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EXPLORING THE BIOPESTICIDAL POTENTIAL OF *SONCHUS ARVENSIS* L. FOR SUSTAINABLE AGRICULTURE AND CROP PROTECTION

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ABSTRACT

Sow thistle (*Sonchus arvensis* L.) contains bioactive compounds with natural defense mechamism against pests and pathogens, making it a potential source for developing biopesticides. In present studies, advanced instrumental analysis methods, including Gas Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC), and spectrophotometry, were employed to analyze the composition of sow thistle extracts. Key compounds such as phenolics, flavonoids, rutin (0.2 µg/ml), quercetin (1.02 µg/ml), kaempferol (0.7 µg/ml), and ferulic acid (9.2 µg/ml) were identified. The extract demonstrated significant antifungal activity, inhibiting Fusarium oxysporum by 72.5% and Alternaria alternata by 78.8%, both major plant pathogens. Additionally, herbicidal effects were observed, with inhibition rates ranging from 29% to 95% on rapeseed germination. These findings highlight the potential of *Sonchus arvensis* as a sustainable biopesticide. Further research will optimize extraction methods for its use in eco-friendly pest management solutions.

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INTRODUCTION

Agriculture is a crucial sector in Mongolia playing a significant role in food production. The country's weather extremes, soil characteristics, and sparsely populated areas enable the cultivation of safe, organic food. However, plant-damaging organisms present a challenge to producing ecologically clean and health-safe food. Utilizing natural products to manage these pests and minimize their damage is considered a safer and less risky approach. Moreover, in Mongolia, chemical pesticides are expensive, have limited variety, and are not readily available. *Sonchus arvensis*, commonly known as Sow Thistle, is native to Europe and Asia. This perennial weed propagates through both seeds and creeping roots. Notably, it exhibits high reproductive potential, with individual plants producing an impressive seed count ranging from 19,000 to 30,000 seeds per plant (Tserenbaljid, 2002). This plant boasts a plethora of biologically active compounds, with foreign researchers confirming its noteworthy attributes, including antioxidative, antibacterial, antifungal, and allelopathic properties (Bashir *et al.*, 2018). The scientists of our country have determined that in terms of chemical composition, the above-ground part of the plant contains carotenes, vitamin C, tartaric acid, triterpene compounds, invertose, inositol, choline,

alkaloids, inositol, mannitol, taraxasterol, lactucerol, mainly a large amount of inulin in the roots, and the content of oil containing stearic and palmitic acids in the seeds is 31.5% (Volodya, 2014; Batish *et al.*, 2007). Sow thistle (*S. arvensis.*L) contains many biologically active substances, among which phenolic compounds are highly active. The sow thistle has an allelopathic or bioherbicidal effect. Allelochemicals with herbicidal activity are classified into 2 groups: phenolic compounds and terpene compounds. Sow thistle (*S. arvensis.L*) belongs to the category of phenolic compounds due to its high tannin and ferulic acid content (Moosavi *et al.*, 2011; Purevjargal *et al.*, 2022). Weeds are quite problematic for agroecosystems. There is an urgent need to study the results of extracting total phenolic compounds, tannin and ferulic acid from the sow thistle against weeds based on its chemical composition. In this way, it will be a work of great importance for the natural control of weeds in agricultural fields, the environment, food safety, and human health. It is necessary to conduct research on the biologically active substances of this plant and to study the disease-causing fungi and allelopathic activity in the plant (Tserenbaljid, 2002).

Allelochemicals stand as pivotal elements in sustainable agricultural practices, wielding significant efficacy in the regulation of weed populations (Tserenbaljid, 2002). The potential application of allelopathy in agriculture is a focal point of extensive research (Kong *et al.*, 2006; Hickman *et al.*, 2023). Utilizing allelochemical-producing plants in agriculture yields notable suppression of weeds and various pests. Certain plants have been observed to decrease the germination rate of other plants by up to 50% (Kato-Noguchi and Tanaka, 2003). Current research is centered on investigating the interactions between weeds and crops, crops and weeds, as well as inter-crop interactions (Kong *et al.*, 2008; Khanh *et al.*, 2005). This research advances the potential utilization of allelochemicals as growth regulators and natural herbicides, contributing to the promotion of sustainable agriculture (Chen *et al.*, 2008). Agricultural practices could be optimized by harnessing the potential of allelochemical-producing plants (Kaiser, 2016). When utilized with precision, these botanical specimens confer pesticidal, herbicidal, and antimicrobial properties to crops. In Mongolia, all herbicides are imported, and previous research on allelopathy has been nonexistent. Our study inaugurates the investigation of allelopathy in indigenous flora and pioneers bioherbicide research within Mongolia's agricultural landscape.

MATERIALS AND METHODS

Preparation of Extract

The dried plant material was meticulously ground into a fine powder using a sample grinder. Subsequently, the powdered material was subjected to methanol extraction at a ratio of 1:5 (plant material weight to methanol volume) for 24 hours. An ultrasonic bath was employed for 6-7 cycles to enhance the extraction efficiency.

Following the extraction process, the resulting solution was carefully filtered to remove any particulate matter. The filtrate was then subjected to concentration via distillation, maintaining a constant temperature of 50°C while utilizing a vacuum evaporator. This step was crucial for removing the solvent and isolating the active compounds.

The concentrated extract obtained was stored in a refrigerator at a temperature of -4°C to preserve its integrity and bioactive properties (Khan, 2012).

Preparation of Standard Solutions

A 99% pure methanol was used to prepare the standard solution of flavonoids. 25 mg of the analytical standards of quercitin, rutin, apigenin and kaempferol were weighed and dissolved in 25 ml of methanol to prepare a 1000 ppm stock solution.

Preparation of Working Standard Solutions

Working standard solutions were meticulously prepared from stock solutions with an initial concentration of 1.0, 2.5, 5.0, 10, and 12.5 parts per billion (ppb). These solutions were derived from high-purity standard substances of quercitrin, kaempferol, and apigenin, exhibiting a purity level of 97-99%.

These standard solutions were analyzed using a high-sensitivity liquid chromatography instrument, operating at specific wavelengths of 272 nm and 310 nm. A C18 column, measuring 250 mm in length and 4.6 mm in diameter, with a 5 μm particle size, was employed in the chromatographic separation process. The separation procedure was performed over a 50-minute to ensure a thorough analysis of the standard substances (Seal, 2016). Through this rigorous analytical approach, the individual standard substances, namely quercitrin, kaempferol, and apigenin, were accurately identified and quantified.

Biochemical Composition and Bioactive Substances in Concentrated Plant Extract

The biochemical composition of the concentrated plant

extract was comprehensively analyzed using a Perkin Elmer Clarus 680 Gas Chromatograph/Mass Spectrometer, a sophisticated instrumental analysis method. This state-of-the-art analytical technique provided precise insights into the constituents of the extract.

The content of critical compounds, including rutin, quercetin, kaempferol, apigenin, and ferulic acid, was quantitatively assessed by comparing them to reference standard substances with a purity of 99%. This rigorous standardization ensured the results' accuracy and relevance for various applications.

The analytical methods applied for this determination followed internationally recognized protocols and methodologies, incorporating a high-sensitivity liquid chromatography instrument. This modern and advanced method allowed for an in-depth examination of the plant extract, adhering to the highest scientific standards and providing reliable data.

Determination of Total Phenolic Compounds and Flavonoid Contents

The concentration of total phenolic compounds was ascertained using the reaction of the Folin-Ciocalteu reagent with phenolic and polyphenolic compounds, forming a distinctive blue colour. To perform this analysis, 0.5 mL of a 10% methanol extract, 0.5 mL of 10% Folin-Ciocalteu reagent, and 2 mL of 7.5% sodium carbonate were combined and maintained at 50°C for 10 minutes. The intensity of light absorbance was then measured at a wavelength of 765 nm using a spectrophotometer.

Total flavonoid content determination was based on forming a colour complex when flavonoids interact with aluminium chloride (AlCl3). This method involved the addition of 1 mL of a 10% methanol extract, 0.2 mL of aluminium chloride (AlCl3), and 5.8 mL of distilled water. After allowing the mixture to stand at room temperature for 30 minutes, the absorbance of light was measured at a wavelength of 415 nm using a spectrophotometer. The obtained values were compared to a reference curve constructed from a standard quercetin solution.

These precise and established methods enable the accurate quantification of both total phenolic compounds and total flavonoid content, offering valuable insights into the sample's potential health-promoting and antioxidant properties (Aryal *et al.*, 2019).

***In vitro* Screening of Antifungal Activity**

The compound's antifungal properties were evaluated by a modified growth rate assay method (Ichinkhorloo, 2016). Fungi were cultured on Potato Dextrose Agar (PDA) medium for 48 hours and transferred to Petri dishes for further testing. The compounds under investigation were initially dissolved in Dimethyl Sulfoxide (DMSO) to create a stock solution with a 1.0 µg/mL concentration. This stock solution was then diluted with water to produce a mixture containing 5% DMSO and water. The 1.0 µg/mL stock solution was combined with a sterilized PDA medium to achieve a final 100 µg/mL concentration. The resulting mixture was aseptically dispensed into Petri dishes within a laminar flow chamber.

As a treated control, Sanguinarine chloride was prepared at a 1.0 µg/mL concentration in a 5% DMSO-water solution. For the untreated control, a 5% DMSO-water solution was used. After the medium in the Petri dishes was partially solidified, fungal discs measuring 5 mm in thickness and 4 mm in diameter were obtained from previously subcultured Petri dishes and placed in the centre of the semi-solid medium. The Petri dishes were then incubated at 28°C for 72 hours. The entire experiment was conducted in triplicate.

The diameters of the inhibition zones, measured in millimetres, were recorded in three directions. Growth inhibition rates were calculated using the formula below and reported as mean values with standard deviations:

$$\text{Growth Inhibition Rate (\%)} = \frac{(dc - d0) - (ds - d0)}{(dc - d0)} \times 100$$

dc: The diameter of the inhibition zone in the control group

d0: The diameter of the inhibition zone in the untreated group

ds: The diameter of the inhibition zone in the tested sample

The variables used in the formula are dc: the diameter of the inhibition zone of the control group, d0: the diameter of the inhibition zone of the untreated group, and ds: the diameter of the inhibition zone of the tested sample. It compares the inhibition zone of the sample with the inhibition zone of the control group and untreated group to find the percentage of growth inhibition of the sample.

Allelopathic Effects on Rapeseed (*Brassica napus*) and Wheat (*Triticum aestivum* L.) Seeds

To assess the allelopathic effects, we prepared

concentrated extracts of sow thistle at concentrations of 1%, 3%, and 5%. Two varieties of rapeseeds, namely black and yellow, along with wheat seeds, were subjected to watering with the sow thistle extract. The germination tests were conducted with three repetitions and compared to a control group.

A meticulous process was followed for seed sterilization. Seeds were initially soaked in distilled water for 6-8 hours. Subsequently, they were rinsed with a soapy solution until no more foam was generated. Each Petri dish used for the germination tests was sterilized with a 1% sodium hypochlorite (NaOCl) solution. The germination progress was monitored continuously over a 120-hour duration (Bashir *et al.*, 2018).

RESULTS

We prepared a concentrated extract from the sow thistle plant and conducted a comprehensive analysis, comparing the results to standard curves of gallic acid and quercetin using a spectrophotometric method. Additionally, we employed advanced instrumental analysis techniques, including gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC), to assess the content of total phenolic compounds, total flavonoids, and the biochemical composition of the extract. This rigorous analysis included quantifying specific types of flavonoids and ferulic acid, following internationally recognized methods.

By combining these modern instrumental approaches with well-established biochemical methodologies, we obtained a thorough understanding of the bioactive constituents within the sow thistle extract, contributing valuable insights to phytochemistry and bioactive compound analysis.

Quantification of total Phenolic Compounds and Flavonoids of Sow Thistal Extract

We quantified the total phenolic compounds by comparing our results with a reference curve of gallic acid standard solutions (ranging from 200 mg/mL to 6.25 mg/mL). Similarly, we determined the total flavonoids by comparing our findings with a reference curve of quercetin standard solutions (ranging from 50 mg/mL to 1.5625 mg/mL).

Our analysis revealed that the sow thistle extract contained 1.08 mg of total phenolic compounds per gram of the extract, as determined through comparison with the gallic acid reference curve. Additionally, the total flavonoid content was found to be 0.102 mg per gram of the extract, as determined by comparing with the quercetin reference curve, as presented in Table 1.

Table 1. Total phenolic compounds and total flavonoid content.

	Total Phenolic Compounds (mg GAE/g)	Total Flavonoid Content (mg QE/g)
Sow Thistle	1.08	1.05

GAE - Gallic Acid Equivalent, QE - Quercetin Equivalent

Biochemical Composition Analysis of Sow Thistle Extract

The concentrated extract obtained from the sow thistle (*Sonchus arvensis*) was subjected to comprehensive analysis using Gas Chromatography and Mass Spectrometry (GC/MS), as illustrated in Figure 1. Following established protocols, the sow thistle extract was meticulously analyzed through Gas Chromatography Mass Spectrometry (GC-MS) and compared against an extensive chromatogram database. This analysis enabled the identification of bioactive compounds with antioxidative and anti-inflammatory properties. Notably, alkaloids, fatty acids, and triterpene compounds were among the critical constituents detected.

Quantification of Specific Flavonoids and Ferulic Acid in Sow Thistle Extract

We quantified the content of select flavonoids, namely quercetin, kaempferol, apigenin, and rutin, as well as the primary active compound, ferulic acid, in the concentrated extract of sow thistle. This was achieved by diluting standard substances of these compounds, prepared initially at a concentration of 1 mg/ml, to 100 µg/ml. The content was then determined using high-performance liquid chromatography (HPLC), as outlined in Table 2.

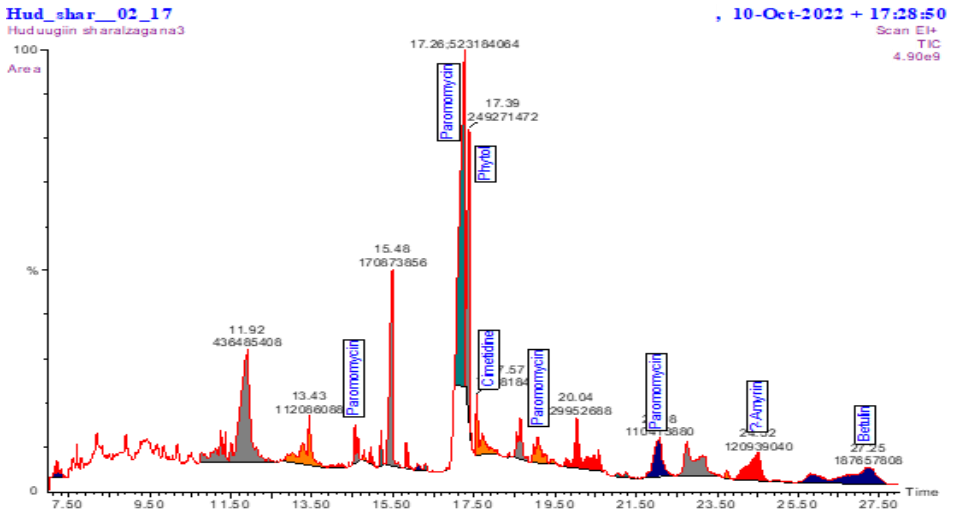

Figure 1. Biochemical composition and volatile compounds revealed by GC/MS in the concentrated sow thistle extract.

Table 2. Content of select flavonoids and ferulic acid.

Flavonoid	Sample Content (µg/ml)	Other Researchers' Range (µg/ml)
Quercetin	1.02	0.78 - 1.35
Kaempferol	0.7	0.947
Apigenin	ND	1.23
Rutin	0.2	0.457
Ferulic Acid	7.568	7.568 - 8.92

ND - Not Detected

Through high- performance liquid chromatography (HPLC), the content of ferulic acid in the sow thistle extract was determined to be 7.568 µg/kg. Apigenin was not detected in the extract. In comparison, quercetin /Quercetin is known to possess antimicrobial activity against bacteria, fungi, and viruses/ content was measured at 1.02 µg/ml, kaempferol at 0.7 µg/ml and rutin at 0.2 µg/ml.

Antifungal Activity of Sow Thistle (*S. arvensis*) Extract

An S concentration of 100 µg/mL could inhibit fungal growth in a laboratory setting and be evaluated using the linear growth rate method. Sow Thistle was used as the control substance. The results of this evaluation are illustrated in Figure 2.

In the presence of *Fusarium oxysporium*, the fungal pathogen cultured in a PDA (Potato Dextrose Agar) medium supplemented with sow thistle exhibited pronounced inhibition rates. Specifically, 51.1% and 72.5% inhibition rates were observed at 1 mg/ml and 5 mg/ml, respectively, over a one-week incubation period. Similarly, when dealing with *Alternaria alternata*, the pathogenic fungi cultured under the same conditions in PDA/sow thistle medium demonstrated inhibition rates of 36.9% and 78.8% at 1 mg/ml and 5 mg/ml, respectively, 7 days (Table 3).

These findings highlight the potent antifungal activity attributed to sow thistle flavonoids. The discernible inhibitory effects, particularly against *Fusarium oxysporium* and *Alternaria alternata,* underscore the potential utility of sow thistle flavonoids as biocontrol agents against these pathogenic fungi.

Allelopathic Impact of Sow Thistle (*S. arvensis*) Extract on Germination of Rapeseed (*Brassica napus*) and Wheat (*Triticum aestivum*) Seeds

To evaluate the allelopathic effects of sow thistle extract, concentrations of 1%, 3%, and 5% were meticulously prepared from the concentrated sow thistle extract. Subsequently, the sow thistle extract treated two distinct varieties of rapeseed (black and yellow) and wheat seeds. The germination tests were conducted with three repetitions for each concentration, and the results were systematically compared with a control group.

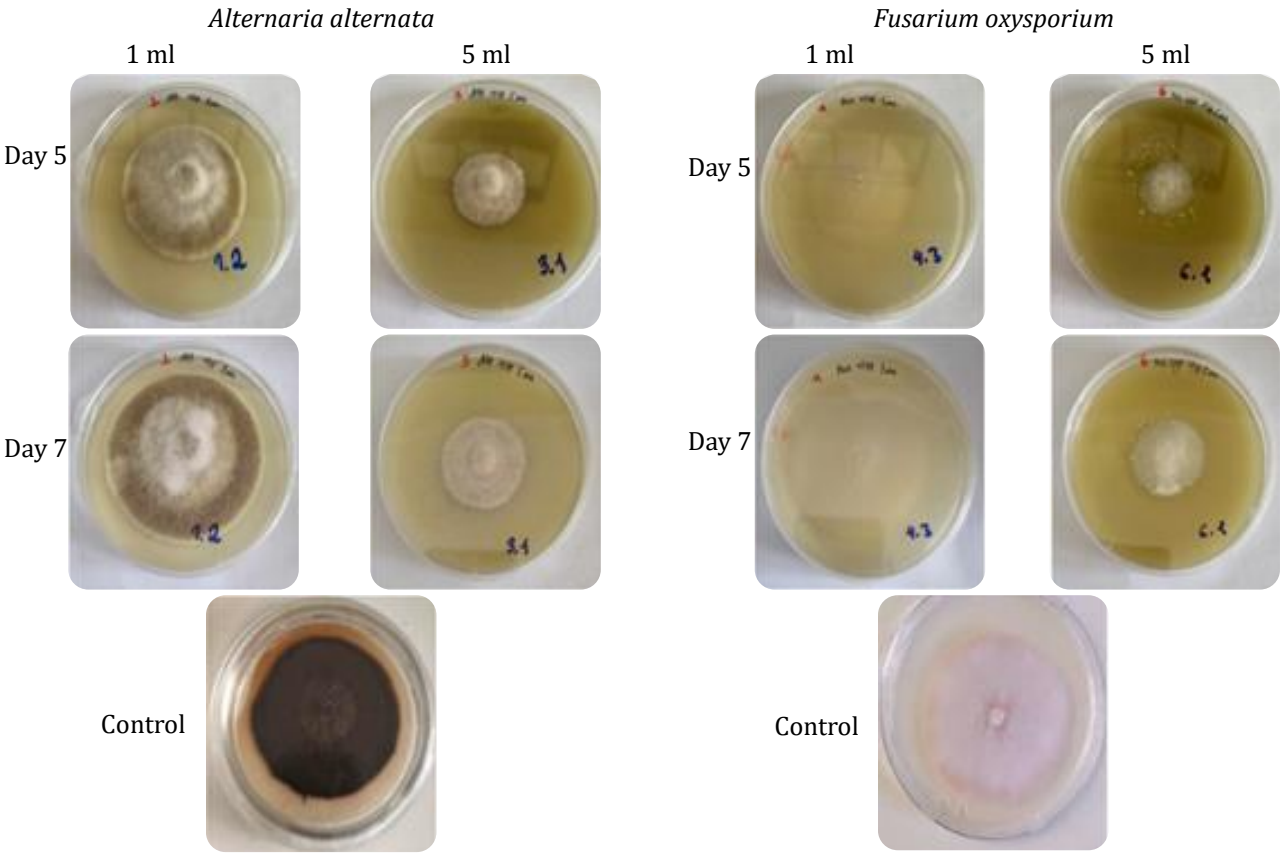

Figure 2. Inhibition of *Alternaria alternata* and *Fusarium oxysporium* by Antagonistic Sow Thistle on PDA at 25 °C for 5 and 7 Days.

Table 3. Antifungal activity of sow thistle extract.

Fungus	Extract, 5 days (%)		Extract, 7 days (%)		DSMO, seven days (%)	
	1 mg/ml	5 mg/ml	1 mg/ml	5 mg/ml	1 mg/ml	5 mg/ml
Fusarium oxysporum	51.1	72.3	37.7	72.5	7.4	ND
Alternaria alternata	36.9	71.9	48.5	78.8	7.1	ND

For seed sterilization, a thorough procedure was followed. The seeds were initially soaked in distilled water for 6-8 hours. Following this, they were meticulously rinsed with a soapy solution until no further foam formation occurred. Subsequently, 50 seeds were evenly distributed in each Petri dish and thoroughly sterilized with a 1% sodium hypochlorite (NaOCl) solution. Over 120 hours, the germination process was closely monitored and recorded.

This study methodically investigated the allelopathic interactions between sow thistle extract and the germination of rapeseed and wheat seeds, providing invaluable insights into the potential phytotoxicity of sow thistle in these agricultural contexts.

The sow thistle extract notably affected the germination of black and yellow rapeseed seeds. In the 1% extract, black rapeseed germination was inhibited by 28.8%, while yellow rapeseed germination was inhibited by 66.2%. In the 3% extract, these inhibitions increased to 38.3% and 83.4% for black and yellow rapeseed, respectively. The highest inhibition was observed in the 5% extract, with black rapeseed germination inhibited by 75.6% and yellow rapeseed germination inhibited by 95.5% (Table 4).

In contrast, the germination of wheat seeds remained relatively stable. In the control group, 20% of wheat seeds germinated; in the 1% extract, this decreased to 10.8%. The 3% extract showed a slight improvement, with 19.4% germination, but it decreased to 10.8% in the 5% extract. These results indicate that sow thistle

extract did not significantly affect the germination of wheat seeds. The most pronounced allelopathic effect was observed in the 5% sow thistle extract, particularly concerning yellow rapeseed.

Table 4. Allelopathic effect of sow thistle extract on rapeseed and wheat seeds.

Extract	Suppressed percent %			
	Control	1%	3%	5%
Wheat	20	10.8	19.4	10.8
Rapeseed (black)	0	28.8	38.3	75.6
Rapeseed (yellow)	0	66.2	83.4	95.5

DISCUSSION

The use of sow thistle in plant protection has been relatively underexplored in scientific literature. However, due to its prevalence as a weed in Mongolia, several textbooks provide valuable insights into its morphology, physiology, chemical composition, distribution, and control methods. Notably, Volodya Ts., in Methodology of Using Mongolian Medicinal Plants in Hospitals (Purevjargal *et al.*, 2022; Zhang *et al.*, 2009), highlighted significant levels of carotenes, vitamin C, tartaric acid, triterpene compounds, invertose, inositol, choline, alkaloids, mannitol, taraxasterol, lactucerol, and substantial quantities of inulin in the above-ground parts of the plant. The presence of 31.5% fatty oils containing stearic and palmitic acids in the seeds aligns with our biochemical composition findings.

Wahyuni *et al.* (2023) analyzed the flavonoid content and antioxidant properties of *S. arvensis*, preparing extracts in methanol, n-hexane, chloroform, and ethyl acetate. The methanol extract exhibited the highest concentration of biologically active substances and flavonoids. Similarly, in our research, the methanol extract showed quercetin at 0.78 µg/ml, rutin at 0.457 µg/ml, and kaempferol at 0.94 µg/ml.

Our research also analyzed the biochemical composition of the concentrated extract of Mongolian saffron using high-performance liquid chromatography (HPLC). The analysis revealed quercetin at 1.02 µg/ml, kaempferol at 0.7 µg/ml, rutin at 0.2 µg/ml, and ferulic acid at 7.568 µg/ml. Bashir *et al.* (2018) similarly investigated the allelopathic activity of *S. arvensis* extracts, demonstrating their inhibitory effects on corn seedling growth under laboratory conditions.

According to the "Organic Agriculture Guide" by Ichinkhorloo (2016), a linear increase in inhibitory activity with rising concentration of allelochemicals was observed, with a 4% extract yielding the most significant results. Water extracts from the stem and leaves of sow thistle were also found effective against wilt disease when applied every 4-6 days. *S. arvensis* demonstrated biological activity against cucumber angular leaf spot, with a 61.6% effectiveness reported (Munkhtsetseg *et al.*, 2020). Thus, plant-derived bio-fungicides are environmentally friendly and economically viable alternatives.

In our antifungal tests against *Fusarium oxysporium*, sow thistle extract inhibited fungal growth by 51.1% at 1 mg/ml and 72.5% at 5 mg/ml after one week. Similarly, *Alternaria alternata* cultures exhibited inhibition rates of 36.9% at 1 mg/ml and 78.8% at 5 mg/ml under the same conditions.

In another study, milk thistle (*Silybum marinum*) extract reduced rapeseed germination by 25%-47.5% when tested with a 1-4% solution (Enkhbulgan G, 2021). In our study, sow thistle extract showed strong allelopathic effects on black and yellow rapeseed germination. The 1% extract inhibited black rapeseed germination by 28.8% and yellow rapeseed by 66.2%, while the 3% extract increased inhibition to 38.3% and 83.4%, respectively. The highest inhibition was seen with the 5% extract, with black rapeseed germination reduced by 75.6% and yellow rapeseed by 95.5%.

These findings highlight the potential of sow thistle for plant protection, providing valuable insights into its chemical composition and its allelopathic and antifungal properties. The results align with prior research, reinforcing the promising applications of sow thistle in agriculture and plant disease management.

CONCLUSIONS

The spectrophotometric analysis of total phenolic compounds and flavonoids indicated concentrations of 1.05–1.08 mg, as determined by comparison with gallic acid and quercetin reference curves. High-performance liquid chromatography (HPLC) analysis of sow thistle extract further identified quercetin at 1.02 µg/ml,

kaempferol at 0.7 µg/ml, rutin at 0.2 µg/ml, and ferulic acid at 7.568 µg/ml. These findings were compared against standard substances. The flavonoids in sow thistle demonstrated notable antifungal activity, with inhibition rates of 51.1% and 72.5% against *Fusarium oxysporium* and 36.9% and 78.8% against *Alternaria alternata* at concentrations of 1 mg/ml and 5 mg/ml, respectively, over 7 days. These results underscore the potential of sow thistle's bioactive compounds as effective biocontrol agents against pathogenic fungi.

Exploring the extraction of biopesticides from widely distributed agricultural weeds in Mongolia offers an opportunity to produce residue-free products that benefit both the environment and human health. Our research aligns with the principles of green chemistry, including waste prevention, safe chemical production, risk minimization, utilization of renewable resources, high atom economy, safe solvent use, improved energy efficiency, biodegradability, and accident risk reduction.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed equally to this work.

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