



Investigation of Lipoxigenase and Xanthine Oxidase Inhibitory Action of *Abies Pindrow* Royle Aerial Parts

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ABSTRACT

To yet, no research has been done on the plant's ability to inhibit xanthine oxidase or lipoxigenase. The plant is used to cure inflammation (related - lipoxigenase enzyme) and jaundice (related - xanthine oxidase) based on traditional applications. As a result, these pharmacological actions of plant will be examined in the current study. The aerial portions of plant were extracted sequentially in a Soxhlet device using solvents that increased in polarity. Petroleum ether's existence of solely lipids was revealed by phytochemical screening; hence it was not tested for any further pharmacological actions. The petroleum ether extract was used to remove fats out of the plant material. Steroids, triterpenoids were detected in the chloroform extract; flavonoids and phenolic compounds were found in the methanol extract; proteins and carbohydrate derivatives were found in the water extract. Using an in vitro standardized spectrophotometric approach, the lipoxigenase and xanthine oxidase inhibitory potential of several crude extracts, including methanol, chloroform, and water extracts, was examined. When compared to the standard lipoxigenase inhibitory drug quercetin, methanol extract of plant aerial parts showed maximum lipoxigenase inhibition action than other tested dose. When compared to the standard xanthine oxidase inhibitory drug allopurinol, methanol extract of plant extract showed strong xanthine oxidase inhibitory activity than other tested extracts. Bioactive methanol extract contained phenols and flavonoids, according to preliminary phytochemical investigations. These natural compounds have been shown to have lipoxigenase and xanthine oxidase inhibitory according to the literature. According to these publications and our findings, flavonoids and phenols are thought to be responsible for current research being done on the aerial sections of *Abies pindrow*.

Key words: *Abies pindrow*, lipoxigenase, xanthine oxidase, flavonoids, phenols.

Introduction

A survey of ethnopharmacological records reveals that *A. pindrow* has been traditionally used in the treatment of diabetes, anxiety, jaundice, cough, phthisis, asthma, bronchitis, fever, catarrh of bladder and inflammation (Chopra et al., 1956; Kirtikar & Basu, 1975; Tiwari and Minocha, 1980; Tripathi et al., 1996; Singh et al., 2000). A review of the literature indicates that this plant's traditional claims have only been partially supported by research conducted on it. To yet, no research has been done on the plant's ability to inhibit xanthine oxidase or lipoxigenase. The plant is used to cure inflammation (linked to the lipoxigenase enzyme) and jaundice (linked to xanthine oxidase) based on traditional applications. As a result, the pharmacological actions of *Abies pindrow* aerial components will be examined in the current study project.

MATERIALS AND METHODS

Collection and identification of plant material

Abies pindrow aerial parts were collected from Gulaba Kothi, Manali, Himachal Pradesh, India at a height of 2000-2100 m in August, 2023. The authentication of the plant material was confirmed on the basis of literature reported and various photographic pictures available online.

Preparation of crude extract / fraction and their phytochemical screening

The plant aerial parts were rinsed with normal saline to remove dirt, dried under sunlight and powdered in a grinder. The solvents were recovered under reduced pressure. All extracts were stored in a vacuum desiccator (Prakash et al., 2015; Kumar et al., 2016; Kumar and Kumar, 2015). The processing of Soxhlet process is presented in figure 1. Crude extracts of plant aerial parts were screened for detection of different classes of phytoconstituents using specific standard reagents (Farnsworth, 1966).

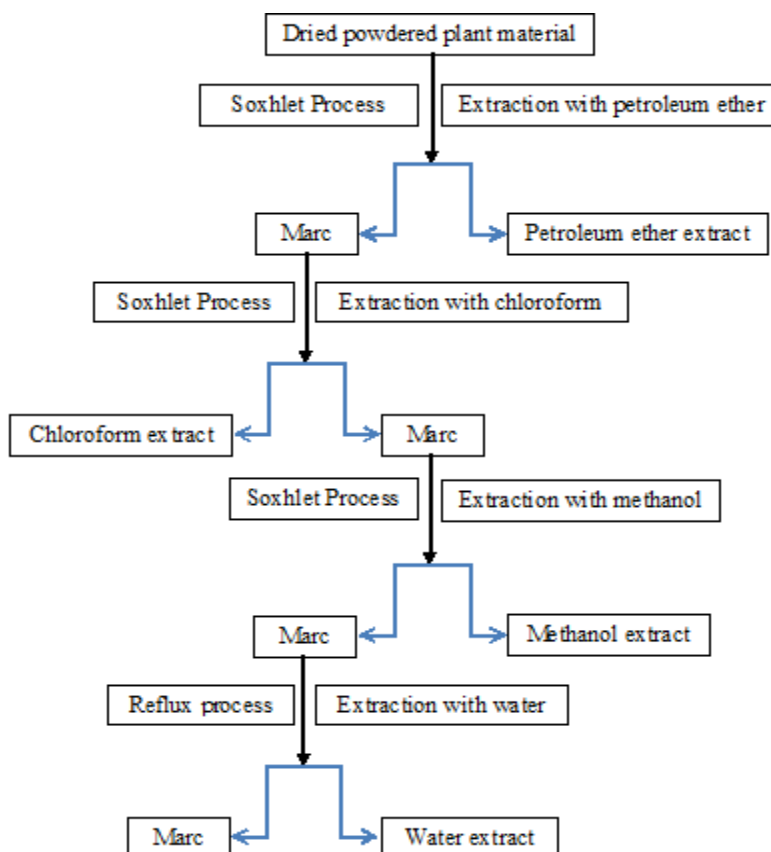


Figure 1: Systematic methodology of Soxhlet extraction technique.

Lipoxygenase inhibition assay

“ Lipoxygenase hindrance movement will be resolved utilizing a spectrophotometric technique. Stock arrangements of the tried examples and quercetin (positive control) at centralization of 10 mg/ml and 100 µg/ml will be set up by dissolving the concentrates and quercetin in DMSO. Sodium phosphate support 2.46 mL (100 mM, pH 8), 10 µL of test tests and 20 µL of soybean lipoxygenase arrangement (167 U/ml) will be blended and brooded at 25°C for 10 min. The response will be then started by the expansion of 10 µL of the substrate as sodium linoleic corrosive arrangement. The enzymatic transformation of sodium linoleic corrosive to frame (9Z, 11E)- (13S)- 13-hydroperoxyoctadeca-9,11-dienoate will be estimated by checking the difference in absorbance at 234 nm over a time of six min utilizing UV-vis spectrophotometer. Another response blend (a negative control) will be set up by supplanting 10 µL tests with 2.47 mL blend of sodium phosphate cradle (5 mL) and DMSO (25 µL) into the quartz (Haq et al., 2004).” All the responses will be acted in triplicates. The level of restraint was determined as:

$$\% \text{ Inhibition} = (A_{bc} - A_s) \times 100 / A_{bc}$$

Where

A_{bc} = This abbreviation represents absorbance of control

A_s = This abbreviation represents absorbance of the tested sample.

Xanthine oxidase inhibition assay

Xanthine oxidase restraint action will be resolved by Sigma convention. Stock arrangements of test tests and allopurinol (as a control) at centralization of 10 mg/ml will be broken down in DMSO. Potassium phosphate support 2.38 mL (0.05 M, pH 7.5), 10 µL of test arrangement and 10 µL of xanthine oxidase arrangement will be blended and hatched at 25°C for 10 min. The response will be then started by the expansion of 100 µL of the substrate as xanthine arrangement. The enzymatic transformation of xanthine to frame uric corrosive and hydrogen peroxides will be estimated at 295 nm utilizing UV-vis spectrophotometer. Another response blend (control) will be set up by supplanting 10 µL of the tried arrangement with 2.39 mL blend of sodium phosphate cushion (5 mL) and DMSO (25 µL) so as to get most extreme uric corrosive development.” The presentation of the test will be confirmed utilizing allopurinol as the positive control (Umamaheswari et al., 2009). All the responses will be acted in triplicates. The level of hindrance will be determined as:

$$\% \text{ Inhibition} = (A_{bc} - A_s) \times 100 / A_{bc}$$

Where

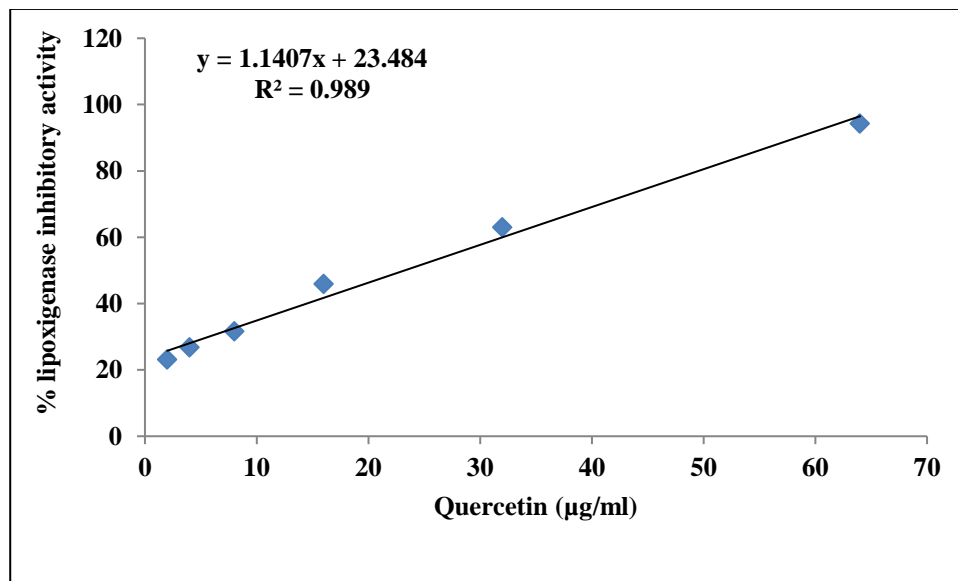
Ab_c = This abbreviation represents absorbance of control

Ab_s = This abbreviation represents absorbance of the tested sample

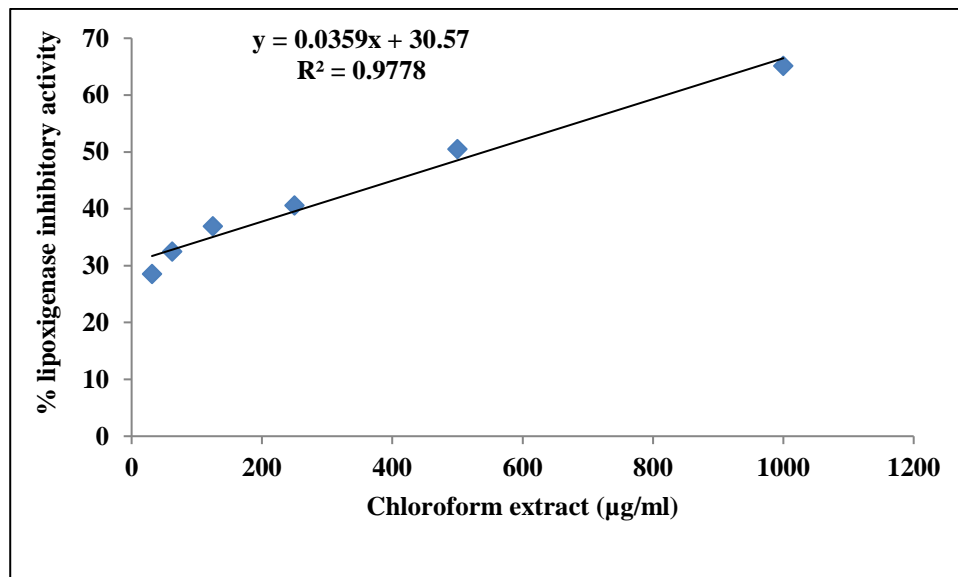
RESULTS AND DISCUSSION

The aerial portions of *Abies pindrow* were extracted sequentially in a Soxhlet device using solvents that increased in polarity: petroleum ether (60–80°C), chloroform, methanol and water extracts and percentage yields of various extracts was found to be 3.45, 4.49, 11.25 and 17.80 % w/w respectively. The petroleum ether extract was used to take the fats out of the plant material. Steroids, triterpenoids were detected in the chloroform extract; flavonoids and phenolic compounds (tannins) were found in the methanol extract; proteins and carbohydrate derivatives were found in the water extract.

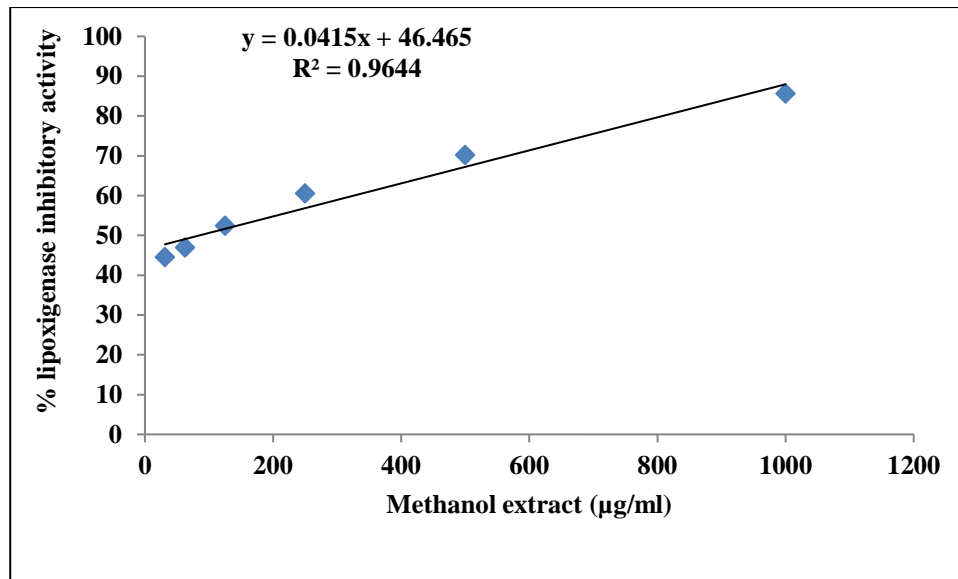
Using an in vitro standardized spectrophotometric approach, the lipoxygenase inhibitory potential of several crude extracts, including methanol, chloroform, and water extracts, was examined (Haq et al., 2004). When compared to the standard lipoxygenase inhibitory drug quercetin (IC₅₀ = 23.24 µg/ml), the methanol extract (IC₅₀ = 85.18 µg/ml) of plant aerial parts showed substantial lipoxygenase inhibition action, followed by the corresponding chloroform extract (IC₅₀ = 541.22 µg/ml) and water extract (IC₅₀ = 888.47 µg/ml). Figure 2 present the lipoxygenase inhibitory activity results.



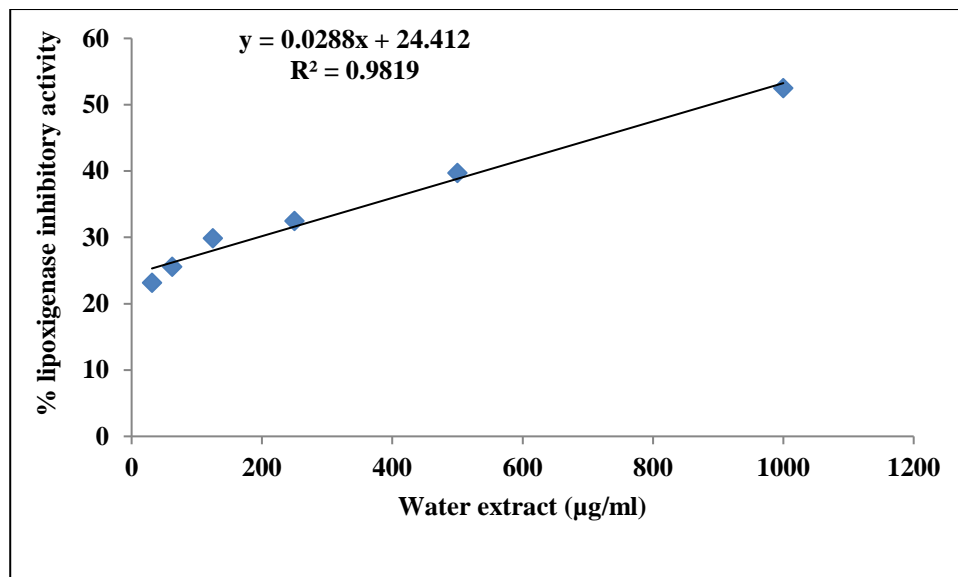
(A)



(B)



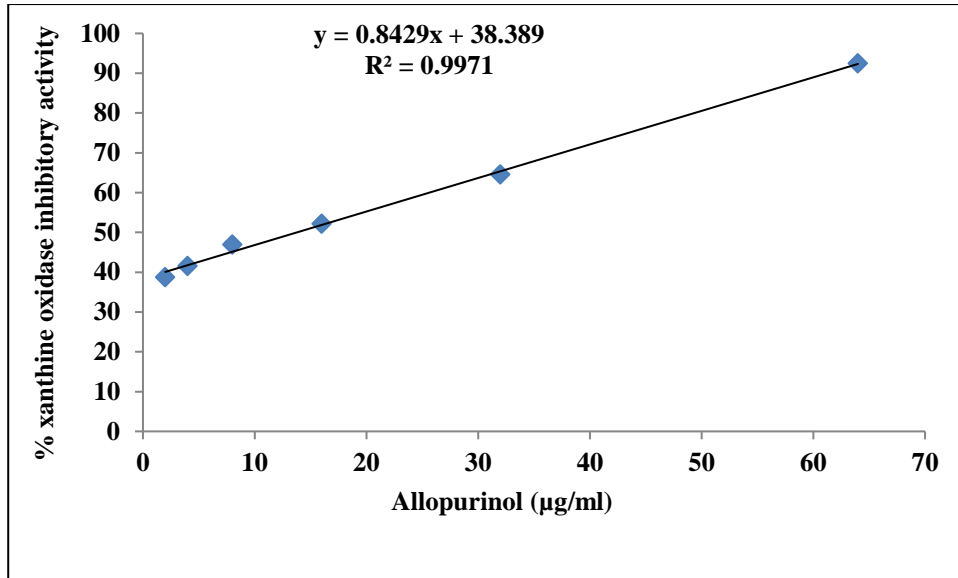
(C)



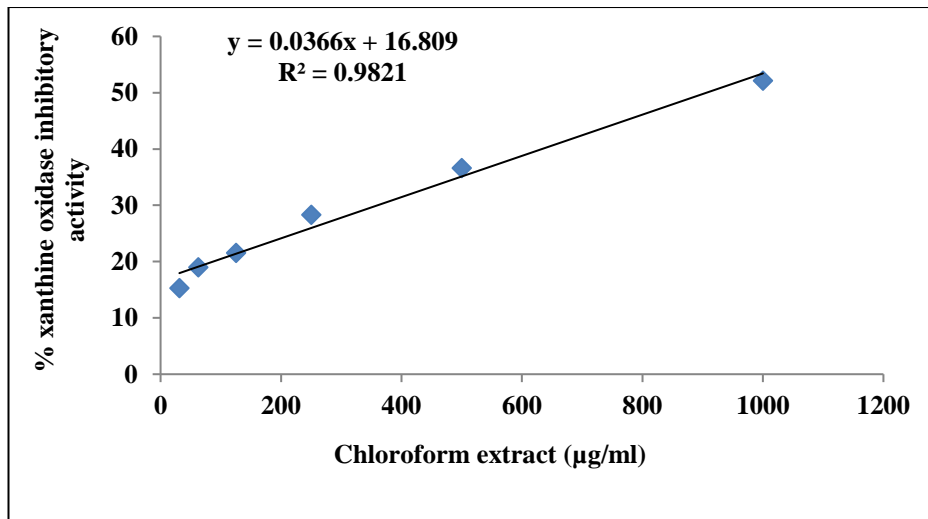
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Figure 2: Graphical representation of % lipoxigenase inhibitory potential of various extracts of *Abies pindrow* aerial parts.

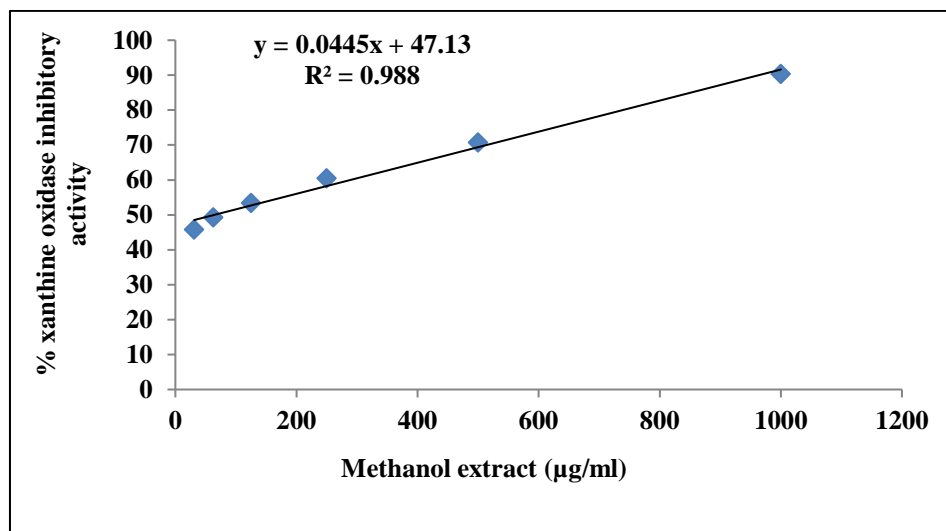
Using an in vitro standardized spectrophotometric approach, the inhibitory activity of xanthine oxidase was assessed in several crude extracts, including methanol, water extracts, and chloroform (Umamaheswari et al., 2009). When compared to the standard xanthine oxidase inhibitory drug allopurinol ($IC_{50} = 13.77 \mu\text{g/ml}$), the methanol extract of the plant extract ($IC_{50} = 64.49 \mu\text{g/ml}$) showed strong xanthine oxidase inhibitory activity, followed by the corresponding water extract ($IC_{50} = 885.60 \mu\text{g/ml}$) and chloroform extract ($IC_{50} = 906.85 \mu\text{g/ml}$). Figure 3 show the outcomes of the xanthine oxidase inhibitory activity.



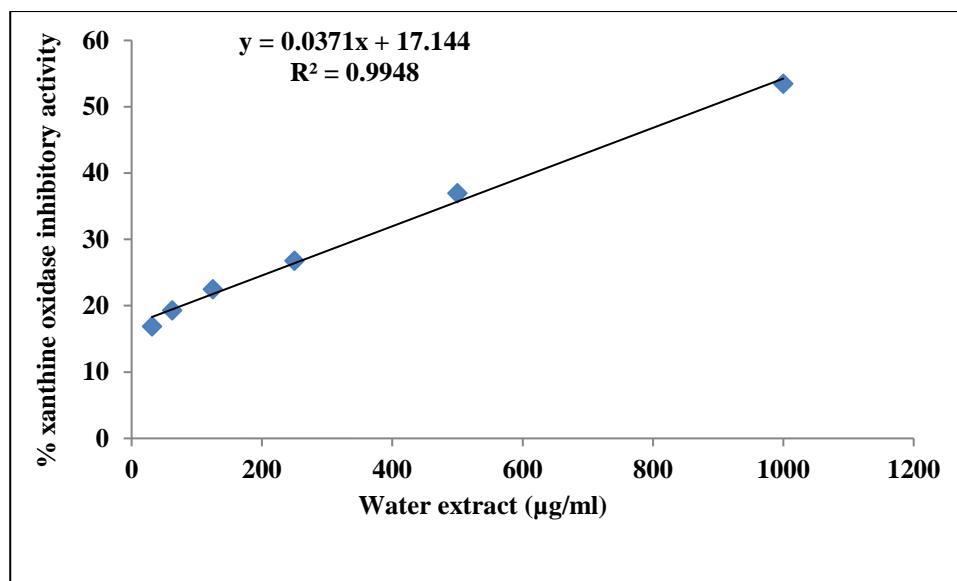
(A)



(B)



(C)



(D)

Figure 3: Graphical representation of % lipoxygenase inhibitory potential of various extracts of *Abies pindrow* aerial parts.

Bioactive methanol extract contained phenolic chemicals and flavonoids, according to preliminary phytochemical investigations. Many flavonoids, including isorhamnetin, rhamnetin, kaempferol, quercetin, myricetin, luteolin, chrysoeriol, diosmetin, and apigenin (Sroka et al., 2017), as well as phenolic compounds, including chlorophenol, guaiacol, and phenol, eugenol (Dohi et al., 1991), have been shown to have lipoxygenase inhibitory activity, according to the literature that is currently available. Numerous flavonoids, including kaempferol, quercetin, and rutin (Isa et al., 2018), apigenin, myricetin, and naringenin (Oskoueian et al., 2011), and phenolic compounds, including gallic acid, salicylic acid, caffeic acid, vanillic acid, and syringic acid (Oskoueian et al., 2011), have been found to exhibit inhibitory activity against xanthine oxidase. According to these publications and our findings, flavonoids and phenolic compounds are thought to be responsible for the current research being done on the aerial sections of *Abies pindrow*.

Acknowledgement

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