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Review Article

In Vitro Evaluation of Antidiabetic Profile of *Abies pindrow* Aerial Parts

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Abstract

Abies pindrow has long tradition of use in natural based medicine system of India for the treatment of diabetes. But traditional claims for antidiabetic activity have not been validated for this plant. Thus, it was considered worthwhile to investigate plant aerial parts for antidiabetic activity. The aerial parts of plant were extracted utilizing solvents in expanding request of polarity viz., petroleum ether, chloroform, methanol, and water. The chloroform, and methanol extracts were subjected to screen *in vitro* antidiabetic activity using starch iodine method via inhibition of α-amylase enzyme. Among different extracts, the methanol extract showed huge antidiabetic action as compared to other tested samples whereas chloroform extract exhibit mild profile of antidiabetic potential. Our preliminary phytochemical investigations showed presence of flavonoids and phenolic compounds in the bioactive methanol extract. Further, exhaustive finding of literature suggested that a number of flavonoids and phenolic compounds are scientifically reported for antidiabetic activity. Thus, finally it can be suggested that these phenolic compounds and flavonoids might be main constituents which are responsible for antidiabetic activity of plant aerial parts. These antidiabetic compounds will be isolated from bioactive methanol extract using chromatographic techniques in future investigations.

Keywords: *Abies pindrow*; Antidiabetic; Flavonoids**;** Himalayan Fir; Phenols

Introduction

Now days, there has been an expanded interest in the utilization of herbal products probably because of their moderately higher accessibility, moderateness, and the apparent lower hazard contrasted with manufactured medications. Around 80% of the total populace by and by utilizes natural medications. The particular utilizations of plant-inferred prescriptions in drug definitions remember their utilization for the treatment of different sicknesses. Herbal medicines potential role in traditional folklore treatment strategies provide a wide remarkable application in human ailments. Customary helpful methodologies assume a potential part in covering fundamental medical advantages. Nature has given an immense materia medica of restorative plants, yet we have efficiently concentrated just a little bit of the world's greenery [1].

Plants are one of the unlimited wellsprings of medication. The plant extracts were initially used as infusion, liniments, syrups, powders and herbal teas [2]. Investigation of compound recognizable proof by different novel innovations made simple to find and disconnect bioactive parts for different remedial advantages, for example, to treat CNS issues, CVS problems, GIT issues, endocrine framework issues and so forth. The essential medical service in the majority of the cultivated social orders relies upon natural medications. In India, despite huge biodiversity, its rich standard mindfulness and legacy of natural prescriptions have

an extremely less commitment to worldwide market. Different therapeutic results of home grown source have been accounted for to have hypolipidemic and hypocholesteremic properties. Reports shows that active principles derived from plants have been demonstrated consistent anti-hyperglycemic and hyperlipidaemic activity [3]. WHO has truth be told suggested the utilization of native plants as an elective cure particularly in non-industrial nations [4]. WHO has recommended explicit norms for the assessment of security, productivity, and remedial advantages of plant extracts. Customary medications have upheld the utilization of spices to treat diabetes and hyperlipidemia. Home grown items are as yet a significant wellspring of new medication disclosures. Fare of natural rough medication from India has been expanded dramatically during the last 2-multi decade.

Review of Literature

Abies pindrow Royle (family-Pinaceae) West Himalayan Fir or Silver Fir or Pindrow fir, is known as 'Morinda' in Hindi and 'Talisapatra' in Sanskrit. It is found mainly in Himalayan deciduous forests, Kashmir, Tehri-Garhwal, and other areas of Northern India, at altitudes of 2,100 to 3,600 m [5].

A. pindrow has narrow pyramidal shape and height of 30 m tall. It is light grey to brown in colour and has fissured bark. Leaves are 1-4 cm long, shiny, dark green, spiral in shape and grooved on upper surface. Male cones have two linear sporangia which have winged microspores. Cones are 1-2 cm long located on auxiliary position and reddish green in colour. Female cones are introverted or in pairs, hardly oblong, 8-12 cm long, have violet purple colour and bear megasporophyll which is 2 cm elongated [6].

Objectives

A. pindrow has been traditionally used in the treatment of diabetes, anxiety disorders, cough, asthma, bronchitis and chest infections [7]. It is used as tonic for bronchitis, haemoptysis, inflammatory conditions, fever and hypoglycaemia [8]. A survey of literature revealed that no antidiabetic related work has been carried out on this potential plant aerial parts till date. Therefore, it was envisaged to undertake detailed *in vitro* antidiabetic activity on *A. pindrow* aerial parts to validate traditional claims of the plant using well developed method known as starch iodine method.

Material and Methods

Collection of plant material

The aerial parts of *Abies pindrow* (1 kg) were collected from Rohtang, Manali, Himachal Pradesh, India in September, 2022. The authentication of the plant material was confirmed on the basis of literature reported and various photographic pictures available online.

Preparation of various extracts of plant material

The plant aerial parts were powdered in a grinder. Dried powdered plant material (250 g) was placed in thimble, made up of fine filter paper. The plant was then extracted in a Soxhlet apparatus with petroleum ether exhaustively till few drops collected from siphoning tube on watch glass did not leave any residue after evaporation. The marc was dried, packed in thimble and extracted exhaustively in a Soxhlet apparatus using chloroform to get chloroform extract. After complete extraction with chloroform, the same procedure was adopted to get methanol extract. The water extract was prepared by boiling the marc of plant material with distilled water for 2 h on a hot plate. The solvents from crude extracts were recovered under reduced pressure using rotary vacuum evaporator. All the extracts of plant were screened for the presence of various groups of phytoconstituents [9].

In Vitro antidiabetic activity studies using starch iodine method

The *in vitro* antidiabetic activity of various extracts and fractions was investigated using starch iodine test via α-amylase enzyme inhibition. The various test samples was made with varied concentrations (50-300 µg/ml), 10 µl of α-amylase enzyme arranged in 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) and was incubated at 37°C for 10 min. At that point dissolvable starch (1%, w/v) was added to every response set and incubated at 37°C for 60 min. 100 µl of 1 M Hydrochloric acid was added to stop the enzymatic response and followed by 200 µl of iodine reagent (5 mM Iodine and 5 mM Potassium Iodide) was added. The shading/colour change was noted and the absorbance was estimated utilizing UV-Vis Spectrophotometer at 595 nm [10] and the IC_{50} was determined. The control response addressing 100% catalyst action did not contain any dried organic product extract or test sample. A dark blue colour indicates the presence of starch; a yellow tone demonstrates the absence of

starch, while a brownish tone shows part of the way degraded starch in the response blend. Within the sight of inhibitors from the test samples, the starch added to the enzyme examine mixture isn't degraded and gives a dark blue shading complex, while no tone complex is created without the inhibitor, demonstrating that starch is totally hydrolysed by α-amylase. Acarbose was utilized as the standard reference. The percentage inhibition of α-amylase enzyme was determined as: [Sample – Control/Sample × 100].

Statistics

The results were expressed as mean ± standard deviation (SD). The test drugs were compared with standard drug and control by one way analysis of variance (ANOVA) followed by Student Tukey's test [11].

Results and Discussion

Abies pindrow aerial parts were extracted exhaustively and successively using solvents in order of increasing polarity. The percentage yields (% w/w) of various extracts of plant aerial parts *viz.,* petroleum ether, chloroform, methanol and water are presented in table 1.

Extract	Yield (% w/w)
Petroleum ether extract	5.25
Chloroform extract	7.40
Methanol extract	10.48
Water extract	13.52

Table 1: Percentage yield of various crude extracts of *Abies pindrow* aerial parts.

All the crude extracts of plant aerial parts were screened for the presence of different classes of phytoconstituents using standard specific chemical reagents. The results of chemical tests of various extracts are shown in table 2.

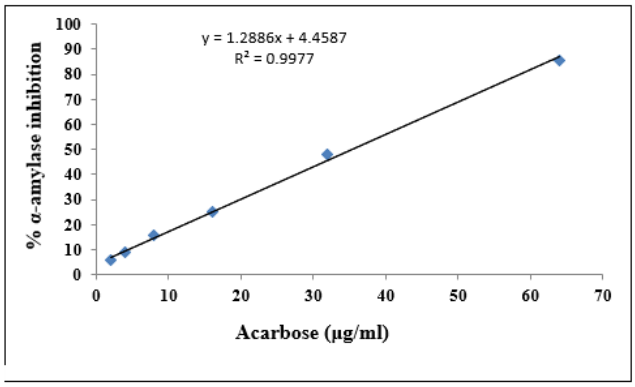
Class of phytoconstituents	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract
Carbohydrates	-	-	-	+
Protein/Amino acids	-	-	-	+
Alkaloids	-	+	-	-
Lipids	+	-	-	-
Anthraquinone glycosides	-	-	-	-
Cardiac glycosides	-	-	-	-
Flavonoids	-	-	+	-
Saponins	-	-	-	-
Tannins/Phenolic compounds	-	-	+	-
Steroids/Triterpenoids	-/-	+/+	-/-	-/-
Coumarins	-	-	-	-

Table 2: Phytochemical screening of various extracts of plant aerial parts.

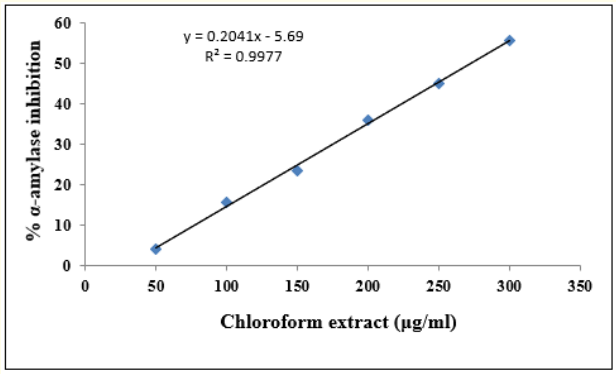
+: Present, -: Absent.

It is clearly evident from table 2 that preliminary phytochemical screening of petroleum ether and water extracts did not show any presence of any main class of phytoconstituents. Therefore, chloroform and methanol extracts were subjected to screen *in vitro* antidiabetic activity using starch iodine method via inhibition of α-amylase enzyme. The antidiabetic activity of various crude samples obtained from plant test drugs was assessed in terms of percentage inhibition of α-amylase enzyme. The results of test drugs were compared with standard drug Acarbose by applying standard statistical analysis method such as one way ANOVA further using by Tukey's test. The methanol extract exhibited maximum antidiabetic activity in term of percentage inhibition of α-amylase enzyme (IC_{50} = 127.40 µg/ml) followed by chloroform extract (IC_{50}

= 272.85 µg/ml). These results are statistically compared with Acarbose standard antidiabetic drug (IC_{50} = 35.34 µg/ml) whereas chloroform extract exhibited mild antidiabetic activity.



(a)



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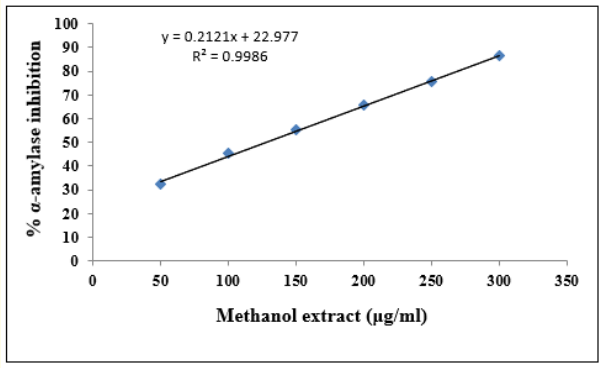

(c)

Figure 1: Graphical representation of antidiabetic activity of various extracts of plant aerial parts using starch iodine method.

Treatment	Concentration (µg/ml)	% α-amylase inhibition (Mean ± S.D.)	IC_{50} values (µg/ml)
Acarbose	2	5.65 ± 0.80	35.34
	4	8.80 ± 1.25	
	8	15.65 ± 2.45	
	16	25.22 ± 2.36	
	32	48.14 ± 3.78	
	64	85.65 ± 3.58	
Chloroform extract	50	4.25 ± 1.11	272.85*
	100	15.80 ± 1.08	
	150	23.45 ± 2.58	
	200	35.98 ± 3.56	
	250	45.14 ± 4.14	
	300	55.59 ± 3.59	
Methanol extract	50	32.47 ± 3.25	127.40*
	100	45.25 ± 4.14	
	150	55.23 ± 3.96	
	200	65.48 ± 4.58	
	250	75.80 ± 5.48	
	300	86.32 ± 5.39	

Table 3: Antidiabetic activity of various crude extracts of plant aerial parts using starch iodine method.

N = 3; *P < 0.05 vs. Rutin; One way ANOVA followed by Student Newman Keul's test.

Our preliminary phytochemical investigations showed presence of flavonoids and phenolic compounds in the bioactive methanol extract. Further, exhaustive finding of literature suggested that a number of flavonoids and phenolic compounds are scientifically reported for antidiabetic activity such as flavonoids – luteolin [12], quercetin, rutin [13], naringenin [14] and phenolic compounds – pinitol [15] have been reported as antidiabetic agents. Thus, finally it can be suggested that these phenolic compounds and flavonoids might be main constituents which are responsible for antidiabetic activity of plant aerial parts.

Conclusion

Thus, finally it can be suggested that these phenolic compounds and flavonoids might be main constituents which are responsible for antidiabetic activity of plant aerial parts. These antidiabetic compounds will be isolated from bioactive methanol extract using chromatographic techniques in future investigations.

Conflict of Interest

There is no conflict of interest.

Financial Assistance

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